The Synthesis and antimicrobial activity of nitropropenyl arenes and related compounds

King H Lo
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School of Applied Sciences
RMIT University
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Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; and, any editorial work, paid or unpaid, carried out by a third party is acknowledged.

King Hei Lo

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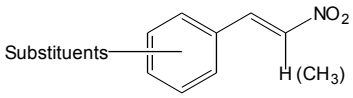
Finally, thanks BioDiem Ltd. for their financial support, without which the project would not have been able to run smoothly.

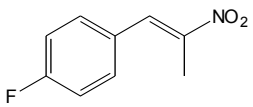
Abstract

This history of antimicrobials is marked with impressive discoveries, the majority of which have their origin in natural products. However in this work, the antimicrobial activity of β-nitrostyrenes and related compounds of synthetic origin is reviewed and investigated with a particular focus on the influence of fluorine functionality.



Various fluorinated *β*-methyl-*β*-nitrostyrene compounds were prepared, their minimum inhibition concentration in cultures of Gram positive, Gram negative and a fungus and their lipophilicity was determined.

Consequently, *1*-fluoro-*4*-(nitroprop-*1*-enyl) benzene [**12c**] was found to have the



1-fluoro-4-(nitroprop-1-enyl) benzene [**12c**]

highest activity against *E. coli*, whereas more lipophilic compounds were more effective against Gram positive bacteria. However compound lipophilicity did not correlate with antimicrobial activity, highlighting the importance of the structure of the antibiotic activity towards the microorganisms studied.

Abbreviations

2-HEAF	2-hydroxyethylammonium formate
α	Alpha
A	Ampere
Å	Angstrom
Ar	Aromatic ring
β	Beta
B. subtilis	*Bacillus subtilis*
BDMI	4-[(E)-2-nitroprop-1-enyl]-1,3-benzodioxole
^{13}C	Carbon-13 NMR
C. albicans	*Candida albicans*
℃	Degree Celsius
COSY	Correlation spectroscopy
CFU/mL	Colony forming units per mL
CH_3COONH_4	Ammonium acetate
$Cu(OTf)_2$	Copper(II) triflate
$CDCl_3$	Deuterated Chlorofrom
CH_3OK	Potassium methoxide
CH_3ONa	Sodium methoxide
CH_3SiSNa	Sodium trimethylsilanethiolate
$(CH_3)_3SiCF_3$	Trimethyl (trifluoromethyl)silane
d	Doublet
dd	Doublet of doublet
DAST	(diethylamino)sulfur trifluoride
DABCO	1,4-diazabicyclo[2.2.2]octane
DBN	1,5-diazabicyclo[5.4.0]nonene-5
DBU	1,5-diazabicyclo[5.4.0]undec-ene-5
DEPT 45	Distortionless Enhancement by Polarization Transfer 45° angle
DEPT 90	Distortionless Enhancement by Polarization Transfer 90° angle
DEPT 135	Distortionless Enhancement by Polarization Transfer 135° angle
Deoxo – Fluor®	Bis (2-methoxyethyl) amino sulfurtrifluoride
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPP-4	dipeptidyl peptidase-4
E	*E* configuration
E. coli	*Escherichia coli*

E. faecalis	*Enterococcus faecalis*
EI	Electron Impact
ESI	Electrospray
EtOH	Ethanol
g	Gram
gCOSY	Correlation spectroscopy with gradient
GC/MS	Gas chromatography coupled to mass spectrometry
GHz	Giga hertz
hr	Hour(s)
^{1}H	Proton-1 NMR
HMBC	Heteronuclear multiple-bond correlation spectroscopy
HSQC	Heteronuclear single-quantum correlation spectroscopy
HPLC	High-performance liquid chromatography
Hz	Hertz
J	Coupling constant
kbar	Kilobar
K_2CO_2	Potassium carbonate
K_D	Partition coefficients
KF	Potassium fluoride
KOH	Potassium hydroxide
LDA	Lithium diisopropylamide
m	multiplet
mmol	Millimole
mL	Millilitre
mPTPB	Mycobacterium protein tyrosine phosphatase B
m/*z*	Mass to charge ratio
M^+	Molecular ion
$MgSO_4$	Magnesium sulfate
MIC	Minimum Inhibitory Concentration
Mtb	*Mycobacterium tuberculosis*
Na_2CO_3	Sodium carbonate
NaOH	Sodium hydroxide
NK-1	Neurokinin-1
NMR	Nuclear magnetic resonance
NaOH	Sodium hydroxide
NK-1	Neurokinin-1
NMR	Nuclear magnetic resonance

p	Pentet
ppm	Parts per million
P.aeruginosa	*Pseudomonas aeruginosa*
PTPs	Protein tyrosine phosphatases
q	Quartet
QSAR	Quantitative structure-activity relationship
r^2	Coefficient of determination
R	Variable group
δ	Delta
δ_H	Chemical shifts for proton -1 NMR
δ_C	Chemical shifts for carbon -13 NMR
s	Singlet
S. aureus	*Staphylococcus aureus*
SARS	Structure-activity relationships
SPAR	Structure-property-activity-relationship
t	triplet
t-BuOK	Potassium tert-butoxide
THF	Tetrahydrofuran
µg/mL	Microgram per millilitre
µL	Microlitre
UV	Ultra Violet
V	Volt
Z	*Z* Configuration

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Chapter 1

1 Introduction

1.1 Resistance of micro-organisms to antibiotics

1.1.1 Background of antimicrobial agents

Antibiotics are chemicals secreted by bacteria and fungi, they can also be synthetic and unnatural to kill or inhibit competitor microbes in the microenvironment and thus are part of microbial self protection[1,2]. At present, many secondary metabolites of bacteria and semi-synthetic antimicrobial agents from natural products have been made even though drug resistant strains has necessitated continuing modifications to the original antibiotic parent compound. From these natural scaffolds medicinal chemists modify structures to create semi-synthetic derivatives with improved properties. Some naturally occurring antibiotics (such as cephalosporins and macrolides) have much more complex chemical structures compared to the synthetic antibiotics (such as sulfa drugs and quinolones).

The first discovery of a successful anti-infective compound was made by a German physician Paul Ehrlich. Ehrlich suggested that to be suitable for therapeutic use, a chemical should be selectively toxic, i.e. show greater toxicity to the target microorganism than to host cells. In 1904 he discovered that the dye trypan red was active against the trypanosome causing African sleeping sickness, and suggested that it could be used therapeutically. Later, Ehrlich successfully synthesized the drug, arsphenamine (Salvarsan) which was used to treat the protozoal disease. Ehrlich, with a Japanese scientist, Sahachiro Hata also found that arsphenamine was active against the syphilis spirochete.

The progress in finding new antimicrobial agents in the next 20 years was slow until the aminoacridine, Proflavine, was introduced in 1934. This drug unfortunately was too toxic to be used against bacterial infections and was used as a disinfectant and antiseptic.[3]

The sulfonamide drugs became the first and the only effective antibacterial agents against systemic bacterial infections until the advent of penicillin G[4, 5]. Gerhard Domagk in the 1930s, showed that the red dye sulfonamidochrysoidine, synthesized by Bayer, completely protected mice against bacterial infections. Later workers at the Pasteur Institute showed that the dye was metabolized by, intestinal micro-organisms to the active form, sulfanilamide. Sulfanilamide (Prontosil), commercialised by Bayer, was the first sulfonamide and it was quickly followed by sulphonamide drugs and the sulfonamides were successfully used to treat *streptococcal* infections.

In 1928 Penicillin was accidently discovered by Alexander Fleming[1, 5] when he noted its inhibitory effect on *Staphylococcus aureus*. Penicillin was the first natural product isolated from the fungus, *Penicillium notatum*. Howard Florey, Ernst Chain and Norman Heatley developed Penicillin as the first antibiotic drug. It was first used therapeutically in 1941[6]. It is active against Gram positive bacteria and the spirochaetes causing syphilis. Howard Florey and his coworkers showed that Penicillin G was more effective in controlling *staphylococcal*, *streptococcal* and *pneumococcal* infections and syphilis. Unfortunately, clinically significant resistance to Penicillin appeared in 1947, but it is still a widely used drug. Many successful semi-synthetic beta-lactam derivatives have been developed from this scaffold. Cephalosporin, which also contains a β-lactam ring, was isolated from a fungus, *Cephalosporium,* in 1948. Similarly, many semi-synthetic derivatives have been developed from this parent compound.

The discovery of the first of the aminoglycoside antibiotics, streptomycin, in the 1940's, extended the anti-bacterial spectrum to include Gram negative bacteria and the tubercle bacillus. More antibiotics were quickly discovered, e.g. the tetracyclines, erythromycin and other marolides.

Overall semi-synthetic derivatives have been developed e.g. sulfa drugs, quinolones, azoles etc, with reference to medicinal chemistry to improve drug scaffolds.

The fluoroquinolones, are a class of synthetic antibacterial agents first synthesized in the early 1960s. Several generations of fluoroquinolones have since been synthesized. They interfere with bacterial DNA gyrase, eventually inhibiting nucleic acid synthesis.

The major classes of antibacterial agents (e.g. β-lactams and tetracyclines group, synthetic sulfonamides and fluoroquinolones[1, 4]) are lead compounds for the synthesis of a new generation of drugs with improved stability, pharmacokinetics and spectrum of activity[7].

Despite the large number of effective antibacterial agents that have been developed, drug resistance occurred quickly for all the major classes of anti-infectives, so there is an urgent need to develop new classes of antimicrobial compounds with diverse microbial targets.

1.1.2 The problems of drug resistance in bacteria

Antimicrobial drugs play an important role in assisting humans in overcoming infections due to pathogenic microorganisms, thus providing successful treatments for microbial diseases. The rapidly increasing emergence of microbial strains resistant to existing antimicrobial agents is a global problem and a serious limitation on the use of antimicrobial chemotherapy and this significant threat particularly is relevant to hospitals. Outbreak infections due to methicillin-resistant *S. aureus*, vancomycin-resistant enterococci and multidrug resistant *Pseudomonas aeruginosa* are increasingly being reported worldwide[8, 9].

Antimicrobial drug resistance is the ability that microorganisms gain to resist biological attack from anti-infective chemotherapeutic agents[4]. Antimicrobial agents are used for medical and veterinary therapy, as disinfectants, antiseptics, agricultural biocides and

food and animal feed additives. The massive amounts of antimicrobial agents being used for many industrial purposes are causing the emergence and spread of drug resistance and giving rise to a rapidly increasing number of pathogenic strains that are resisting treatment with anti-infective agents[4, 5, 10].

1.1.3 Mechanisms of drug resistance

The resistance of bacteria to antibiotics is a natural phenomenon[4, 6]. In nature microorganisms develop mutations or acquire resistance genes to common metabolites from other microorganisms in the environment. Resistance genes are readily transmitted horizontally between related species, and even between unrelated species, so they can spread through microbial habitats, particularly if there is a selection pressure from the presence of industrial antibiotics.

There are a few common mechanisms of drug resistance in bacteria.

(1) Lowered penetration or permeability of drugs into the cell membrane of pathogens. For example Penicillin G is not effective against enteric and related gram negative bacteria as it cannot penetrate the outer membrane[1, 4, 5].

(2) Alteration in a drug's target receptors lowers the binding efficacy of the drug. Vancomycin is no longer effective against *enterococci* because the target for the vancomycin terminal D-alanine-D-alanine in *enterococcal* peptidoglycan has been changed to D-alanine-D-lactate.

(3) Drugs may also be expelled by the pathogen's plasma membrane translocases. Efflux pumps can be specific to one drug as in tetracycline resistance. Multidrug-resistant pumps are relatively nonspecific and can pump many different unrelated drugs out of the cell. Gram negative bacteria such as *E. coli* and *Pseudomonas aeruginosa* contain this type of efflux system[4, 5, 8].

(4) Bacteria can secrete enzymes that modify drugs thereby inactivating them[1, 4, 5]. For example, secretion of bacterial beta lactamases which hydrolyze the β-lactam antibiotics

(e.g. penicillin, cephalosporin) makes them clinically ineffective[5, 10] as the β-lactam ring is the key structural component of these antibiotics.

(5) Bacteria can develop an alternative metabolic/biochemical pathway to make their products[1, 4, 5] and they take up folic acid from their surroundings, therefore they do not need to synthesize folic acid when the pathway to make folic acid is blocked by sulfonamide drugs.

(6) Some antimicrobial agents may not be able to inhibit bacteria if they do not have the structure that antimicrobial agents can target. In other words, the bacteria are naturally resistant to some antimicrobial agents. *Mycoplasmas* bacteria are naturally resistant to penicillins because they do not have a cell wall.

1.1.4 The necessity for new antimicrobial agents

The number of new anti-infective drugs brought to market in the last 20 years has been very low[11, 12]. No new major class antibiotics since the fluoroquinolones have been discovered between 1962 and 2000[1]. The newest naturally occurring antibiotics which have been put into clinical practice were the oxazolidinones in 2000[1]. All the other new agents have narrow spectrum of activity effective only against a few pathogens or of only one type. Another reason is that the development of new antibacterial agents is a costly and time-consuming process before a new drug can be brought to market. For this reason many pharmaceutical companies have stopped or limited their efforts to develop new antimicrobial agents. Only a few pharmaceutical companies are currently active in this field[10]. Scientists have already discovered some major antibiotic cellular targets for drugs to kill or inhibit the growth of microorganisms. Those major cellular targets include: the bacterial cell wall; the bacterial plasma membrane; synthesis of bacterial proteins, bacterial nucleic acids and bacterial metabolism.[1]

Barker has reviewed recent antibacterial drug discovery and structure-based design for the development of new antibacterial compounds[2]. It has been proposed that structure-based design is an excellent tool for designing compounds with increased potency and selectivity. As well as this, a molecular approach can focus chemistry on regions suitable for modification, improving stability or bulk properties such as solubility, without affecting potency. Barker,[2] Bush *et al*.[13] and Projan and Bradford[14] have suggested that new drug development should focus on some new targets for inhibition such as fatty acid synthesis. There is evidence that protein tyrosine phosphatase could be a possible cellular target[7, 15].

1.1.5 Protein tyrosine phosphatase

Protein tyrosine phosphatases (PTPs) exist in both eukaryotic and prokaryotic cells. Scientists have investigated the role of protein tyrosine phosphatase in eukaryotes and have found that many important cell functions such as, cell growth and differentiation, cell motility, metabolism and the immune system. However the discovery of Protein tyrosine phosphatases in bacteria occurred much later, the tyrosine phosphorylation in bacteria is less common and less well investigated. Zhou *et al*.[15] recently investigated protein tyrosine phosphatase B in *Mycobacterium tuberculosis* (*Mtb*). They suggested that mycobacterium protein tyrosine phosphatase B (mPTPB) secreted by *Mtb* might mediate *Mtb* survival in marcophages in the host cell. Thus specific mPTPB inhibitors may help the host cell to enlarge the intrinsic host signaling pathways to eliminate the tuberculosis infection.

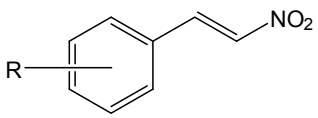
White[7] tested the ability of 4-[(*E*)-2-nitroprop-1-enyl]-1,3-benzodioxole (Compound **7**) to inhibit human and bacterial tyrosine phosphatases by enzymic assay. She stated that nitroalkene compounds related to **7** have been shown to be a competitive, slow and reversible inhibitor of protein tyrosine phosphatase. It was found that **7** showed less inhibitive ability to protein tyrosine phosphatase, which was consistent with the results reported by Park and Pei[16] for related benzyl nitropropene compounds. Both of these

results have suggested that **7** is a less potent inhibitor of tyrosine phosphatases. Thus, White suggested **7** as a potential lead compound to develop anti-infective agents based on PTP inhibition. In other words, the nitroalkenes like nitrostyrene and β-methyl-β-nitrostyrene which have similar chemical structure to **7** are potential compounds for the development of new inhibitors of protein tyrosine phosphatase in bacteria.

1.2 Antimicrobial history of nitrostyrene

1.2.1 Early reports of the antibacterial activity of β-nitrostyrene

Substituted β-nitrostyrenes [structure **1a**] are members of the class of nitroalkenes, and their biological activities have been studied previously for a few strains of bacteria or fungi[17-22].

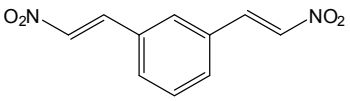


1a

Reports have shown that β-nitrostyrene [**1a**] derivatives have a toxic effect on insects[23, 24] and inhibit the growth of fungi[20, 23, 25]. For example, one of the frequently used fungicides, β-bromo-β-nitrostyrene, has a wide-spectrum of activity against fungi[26]. It may therefore be possible to use this type of compound for the protective treatment of organic materials such as leather. Based on the biological properties of β-nitrostyrene, it could also be used as an antibacterial agent.

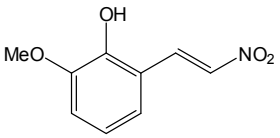
Large numbers of derivatives of β-nitrostyrene [**1a**] were investigated for activity against bacteria by Schales and Graefe[19]. In 1952, they synthesized 55 compounds including 20 new arylnitroalkenes using the Henry reaction and tested their antibacterial properties against the Gram positive bacterium, *Micrococcus pyogenes* var. *aureus*, and the Gram negative bacterium, *Escherichia coli*. The β-nitrostyrene derivatives were synthesized from benzaldehyde and its derivatives having different substituents on the aromatic ring

by reaction with nitromethane using various catalysts. It was shown that, β-nitrostyrene derivatives had activity against both the Gram positive and Gram negative bacteria. The data showed the effectiveness of selected compounds against both types of bacteria especially when substituents such as the methoxy group (-OCH_3) were present on the aromatic ring[19]. However, some compounds were less effective than the parent compound, β-nitrostyrene. They showed that the effectiveness of each compound against *M. pyogenes* was slightly decreased or not affected (for compound **2**) by introducing albumin into the culture medium.



2

Early work by Schales and Graefe indicated that the presence of plasma proteins reduced the biological activity of antibacterial agents[27], but this was not always the case, as they showed that addition of albumin to the culture medium actually enhanced the antimicrobial activity of compounds **3** and **4**.



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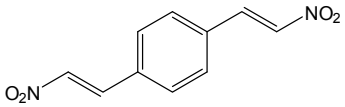

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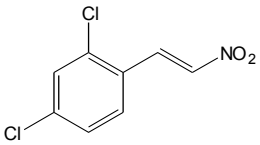
Additional work done by them showed that chlorine substitutuents at the position 4 (*para* to the vinyl group) (compounds **5** and **6**) of the ring showed improved biological activity compared with positions 2 and 3 (data not shown).



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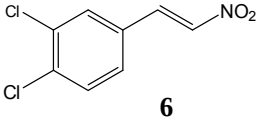


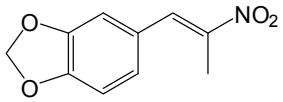
6

Nitroethane was used to form the β-methyl-β-nitrostyrene compounds. The nitropropene compounds were found to be more effective against *Micrococcus pyogenes* var. *aureus*, but were not as effective against *E. coli*. From the published biological activity of β-

nitrostyrene, and other research on nitrostyrene derivatives, it could be concluded that β-nitrostyrene derivatives have potential as antibacterial agents.

1.2.2 Nitrostyrene derivatives as antimicrobial agents

Compound [**7**], is broadly active against a wide range of Gram positive bacteria, Gram negative bacteria, filamentous fungi and yeast.[28, 29]. It is a yellow-colored crystalline compound with melting point of 96°C, is insoluble in water but is soluble in organic solvents such as acetone and ethanol and is stable at room temperature, and to heat, but unstable to UV light on long time exposure. It strongly and reversibly binds to serum albumin[7].

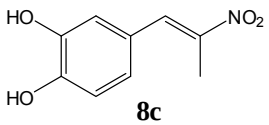


7

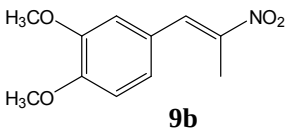
In 1997, Denisenko *et al*.[28] made a series of known nitrostyrene compounds by the Henry reaction in which nitro alkene compounds were synthesized from aromatic aldehydes. Denisenko *et al*. tested these compounds for antimicrobial activity and showed that many compounds had biological activity against the bacterial strains. Further studies on **7** by White confirmed its broad antimicrobial activity[7]. She also showed that **7** does not alter the function of major bacterial targets such as DNA replication, ribosomal function, cell wall synthesis or cell membrane integrity or the synthesis of major fungal targets of cell membrane and cell walls. However, **7** did inhibit protein tyrosine phosphatases in bacteria. The broad spectrum of activity against resistant bacteria, the metabolic targets of **7** in both prokaryotic and eukaryotic microorganisms is sufficiently selective to allow for differential toxicity between microbial and mammalian cells[7].

1.2.3 Recent work on antibacterial activity

Milhazes *et al*.[22], in 2006, synthesized analogues of β-nitrostyrene and β-methyl-β-nitrostyrene derivatives with substituents on the aromatic ring such as hydroxy groups (-OH, compound **8c**), methoxy groups (-OCH_3, compound **9b**) and the methylene dioxy group (-OCH_2O-, **7**) and studied the influence of aromatic substitution patterns on antibacterial activity.

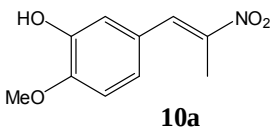


8c



9b

They proposed that these substituents would provide different electronic environments, possibly affecting the antimicrobial activity of these nitrostyrene derivatives. Milhazes *et al*. also mentioned the development of new antimicrobial drugs generally based on the structure-activity relationship (SAR), structure-property-activity relationship (SPAR) and quantitative structure-activity relationship (QSAR) studies[30, 31]. In their investigation of the antimicrobial activity, Gram positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) were used to test their nitrostyrene derivatives. It was found that a methyl group on the β-carbon leads to an increase of the inhibitory effect and the potency was in the range of 2 to 8 fold greater than the parent compound, β-nitrostyrene. The enhancement of activity by the methyl group on the β-carbon was most pronounced on the Gram positive bacteria (e.g. *S. aureus*). The compound 3-hydroxy-4methoxy-β-methyl-β-nitrostyrene [**10a**] gave the best results (MIC 16) against all Gram positive bacteria while the 3,4-dihydroxy-β-methyl-β-nitrostyrene [**8c**] was the most effective (MIC 64) against all the Gram negative bacteria except for *P. aeruginosa*. (Table 1)



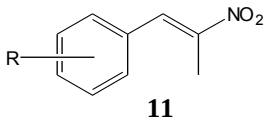
10a

Table 1: Antimicrobial activity of the β-methyl-β-nitrostyrene derivatives against several bacteria[22]

Strain	Minimum Inhibitory Concentration, MIC (mg/L)		
	8c	**9b**	**10a**
Escherichia coli	64	256	128
Pseudomonas aeruginosa	256	256	256
Enterococcus faecalis	64	32	16
Staphylococcus aureus	64	32	16

Furthermore, Milhazes *et al*. found that antibacterial activity did not increase even though changing the polarity of the compounds with aromatic substituents would also change the electronic characteristics and lipophilicity of the compound (they only tested *S. aureus*). They also concluded that β-methyl-β-nitrostyrene derivatives could be potential anti-microbial agents for clinical use.

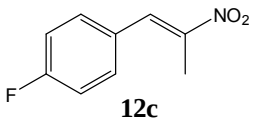
Recent work related to the synthesis and antimicrobial activity of nitrostyrene derivatives was reported by Nicoletti *et al*.[29] They focused mainly on synthesizing β-methyl-β-nitrostyrene derivatives [**11**], and evaluated their antimicrobial activity against a panel of Gram positive bacteria, Gram negative bacteria and fungi.



11

R = variable group

In the nitrostyrene compounds, there were different substituents on the aromatic ring and other novel reactions were carried out to produce pyridine, imidazole, benzoxazole, thiazole and other analogues. According to their results, most compounds had significantly high activity against Gram positive bacteria and fungi. The compound which had the highest activity against Gram negative bacteria was the 4-fluoro derivative, 4-(2-nitroprop-1-enyl)-1-fluorobenzene, **12c**.

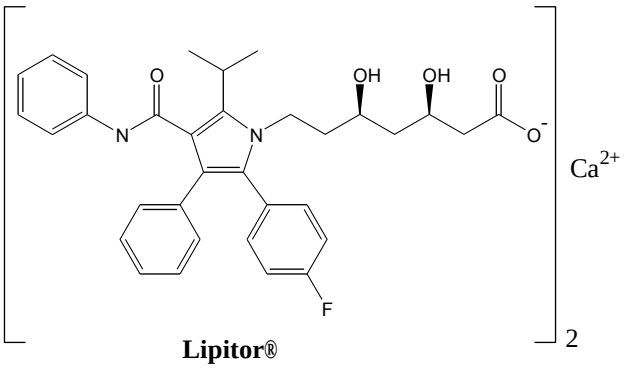


12c

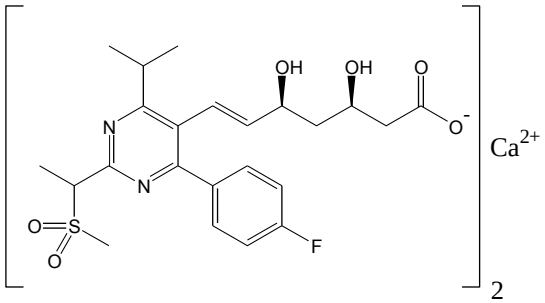
Based on these discoveries, new designs for the synthesis and development of new antibacterial compounds for SAR were continued in this investigation (Masters Program). The Henry reaction was used and compounds were prepared under two different conditions: Method A and Method B (See Chapter 3). The lipophilicity of each compound was measured by determination of octanol-water partition coefficients. The methods of evaluation of antibacterial activity were determined by minimum inhibitory concentration (MIC).

1.3 Fluorine as a substituent

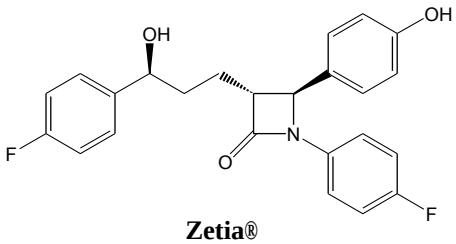
This project, in part, investigated the antimicrobial activity of fluorine substituted nitrostyrene compounds. The 1906 Nobel Prize in Chemistry was awarded to Henri Moissan for his discovery and isolation of the element fluorine. Neil Barrett never received the Nobel Prize, however, in 1962 he was the first to produce xenon fluoride thereby discovering the reactivity of noble gases in forming fluorides. Organic compounds with very stable covalent carbon-fluorine bonds are produced when fluorine atoms or groups are substituted for hydrogen or oxygen. An example is the ubiquitous use of Teflon (polytetrafluoroethylene) coatings in non-stick frying pans. Two fluorine compounds, highlights of medicinal chemistry research in the 1950s, are the anti-inflammatory drug 9α-fluoro-hydrocortisone acetate and the anticancer drug 5-fluorouracil. In recent times, drugs for the treatment of high cholesterol levels include atorvastatin calcium (**Lipitor®**, Pfizer), rosuvastatin calcium (**Crestor®**, AstraZeneca), ezetimibe **(Zetia®**, Merck/Schering-Plough) and fluvastatin sodium (**Lescol®**, Novartis), and all contain one or more fluorine atoms and are amongst the highest selling prescription drugs developed to date. As well as this, compounds with fluorine substitution have been widely used in the manufacture of medicines, agrochemicals and polymers in recent years[32-35].



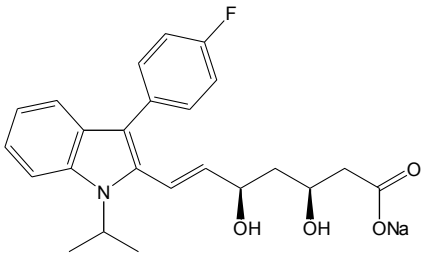
Lipitor®



Crestor®



Zetia®



Lescol®

Some examples of fluorine-containing drugs

Reports have shown that not more than 40 organofluorine compounds from natural products have been isolated, but none of them contained an aryl fluoride in their structure[36, 37]. Previous reviews have shown that the fluorine atom itself is not sterically demanding and has a very small van der Waals radius[38], which is slightly larger in size to the hydrogen atom. Carbon-fluorine bonds are considered the strongest covalent bonds. Fluorine always increases hydrogen bond acidity[39], and the strength of carbon-fluorine bond is high, 439.6 kJ/mol[32, 38-40]. It can also go to higher bond energy of 485.7 kJ/mol[41], Table 2 shows some properties of hydrogen, fluorine, chlorine, oxygen and carbon. The thermal stability could be enhanced due to this bonding energy. When hydrogen is replaced by fluorine, the lipid solubility/lipophilicity or hydrophobicity would be expected to increase biological absorption, although this was not the case for the fluorination of alkanes[39, 42, 43].

Table 2: Some properties of H, F, Cl, O and C

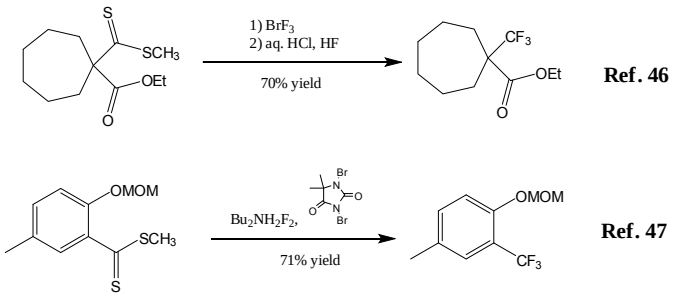
	van der Waals radius (Å)	Electronegativity (Pauling)	Bond Strength with carbon (kJ mol^{-1})
Hydrogen	1.20	2.1	413
Fluorine	1.47	4.0	485
Chlorine	1.75	3.2	339
Oxygen	1.52	3.4	358
Carbon	1.70	2.5	346

Fluorine has the highest electronegativity of all the elements, which means it has a high ionization potential. Therefore it has a higher ability to withdraw electrons from other atoms in molecules towards fluorine, and that modifies the reactivity of compounds containing fluorine (refer to Chapter 2). Fluorine has a noteable leaving group ability, offering the possibility to design mechanism-based enzyme inhibitors[40] and the small covalent radius can facilitate docking with drug receptor(s)[39]. Because of the low F-F bond energy (36.6 kcal/mol or 153 kJ/mol), the strong repulsion of its lone pair electrons, when reacted with other compounds to form high energy bonds, makes reactions of F_2 with other elements or compounds extremely exothermic and often explosive[44]. Other concerns are, for example high reactivity, lack of selectivity and potential toxicity (due to the inability of fluorinated compounds to be metabolized)[33] as well as the risk of free radical initiation during reaction. All these properties make working with elemental fluorine a challenge[44].

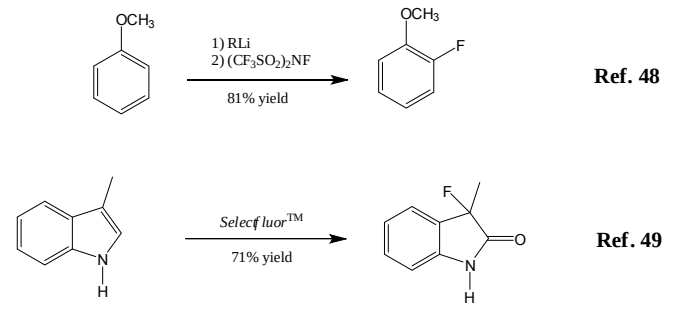
1.4 Fluorine in organic chemistry

Petrik and Cahard[45] and other workers indicated that organic compounds of fluorine are rare in nature whereas laboratory synthesized fluorinated compounds have readily been prepared and research related to fluorine is widespread in chemical sciences. Introduction

of fluorine into an organic molecule can be achieved by using nucleophilic fluorine reagents (Scheme 1)[46, 47] and electrophilic fluorine reagents (Scheme 2)[48, 49].

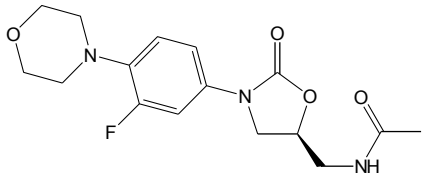


Scheme 1: Examples of introduction of fluorine via nucleophilic reagents



Scheme 2: Examples of introduction of fluorine aromatic rings via electrophilic reagents

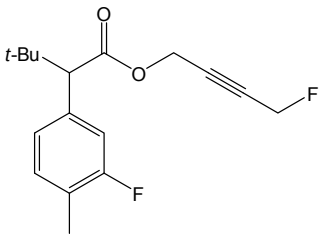
Fluoroorganic chemistry is becoming important in science as it has many applications in chemistry and medicinal chemistry. Fluorine-containing compounds are well known antibacterial agents; for example, a fluorine atom improves the hydrogen bond donor acidity which makes a hydrogen bond with protein and lipid component of biosystem easily to elicit bioactivity in bacteria[33]. In another example Giménez *et al*.[50] showed that a fluorine atom improved drug activity due to the increase in hydrophobicity of the drug. Ismail[51] mentioned that **Linezolid** can be used totreat infections caused by serious Gram positive bacteria and Purser *et al*.[52] gave some examples about recent effective fluorinated antibacterial agents now on the market. Moreover, substitution with fluorine in nitrostyrene in a previous study[53] was shown to enhance the antimicrobial properties of these compounds.



Linezolid

The van der Waals radius of fluorine is approximately 1.47Å and is between oxygen (1.52 Å) and hydrogen (1.20 Å)[51, 54]. It enables fluorine to have the ability to form hydrogen bond. The strong C-F bond enables organofluoride compounds to kill pests harmful to agriculture. There are examples of organofluorides being used as insecticides [**13**[55] and **14**[56]], as arthropodicides [**15**],[57] as herbicides [**16**],[58] as fungicides [**17**[59], **18**[60] and **19**[61]], as pesticides [**20**],[62] as agrochemicals [**21**[63] and **22**[64]] and pharmaceuticals [**23**[65, 66] and **24**[67]] uses. (Full name of compounds in Appendix)

Examples of organofluorides as insecticides, arthropodicides, herbicides, fungicides and pesticides



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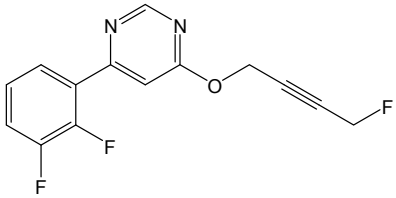

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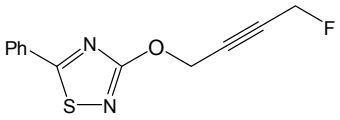

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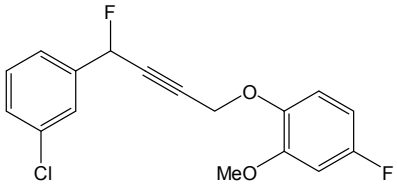

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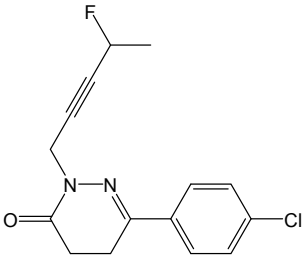

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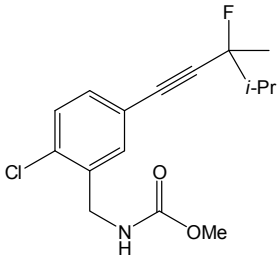

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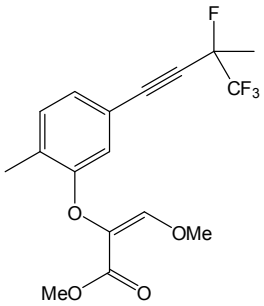

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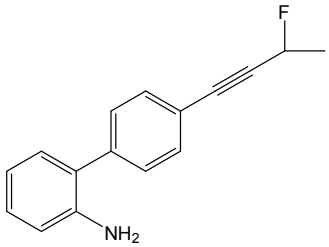

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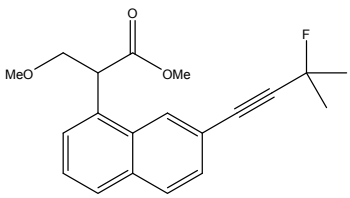
Examples of fluorine containing agrochemicals



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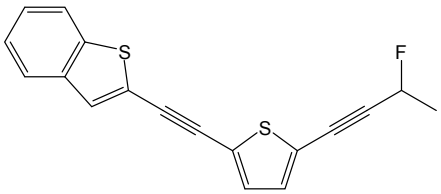

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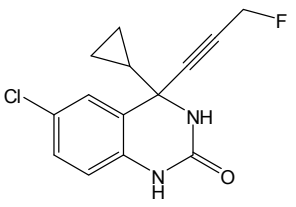
Examples of organofluoride pharmaceuticals



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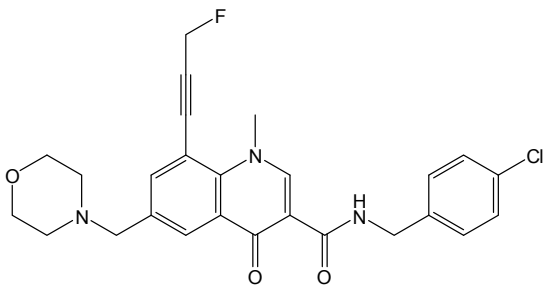
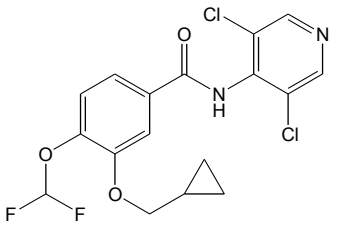


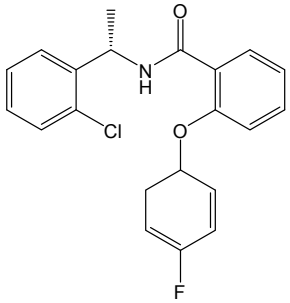
24

Other examples of biologically activity fluorine substituted compounds are the important phosphodiesterase inhibitors **Roflumilast** and *N*-[1-(2-chloro-phenyl)ethyl]-2-(4-fluoro-phenoxy)benzamide [25][51].

Two examples of phosphodiesterase inhibitors



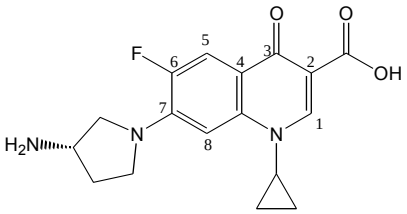
Roflumilast



25

Previous discoveries of fluorine-containing compounds that showed effective antimicrobial properties are exemplified by the fluoro-quinolones and the fluorinated quinazoline derivatives[68, 69]. Fluoroquinazoline has been widely used as a fungicide[70] herbicide[71] and additionally as an antitumor agent[72]. Fluoro-quinolones, first approved in the 1960s, had emerged as a significant class of chemotherapeuric agents[73]. Clairefond *et al*.[73] showed the effect of fluorine at carbon-5, 6 or 8 in a series of compounds that were tested against *E. coli* DNA-gyrase.

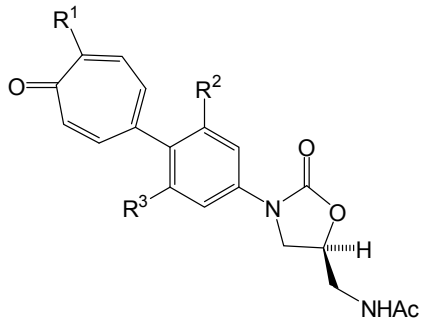
Fluoro-quinolone compound with C-6 fluorine substitution



26

They found that fluorine substitution at carbon-6 [26] or -8 showed enhancements of antimicrobial activity against both Gram positive and Gram negative bacteria. However, fluorine substitution at carbon -5 had markedly decreased activity due to compensatory electronic effects. Koga *et al*. carried out similar experiments but obtained markedly different results in the potency of the compound substituted at carbon-8 *in vitro* (10 fold

less active).[74] The oxazolidinones were other examples given by Barbachyn *et al*.[75] that showed fluorine substitution enhanced antibacterial activity. He referred to the structure activity-relationships of Gregory *et al*.[76] who postulated that reducing the electron density in the phenyl ring of phenyloxazolidinones by substitution of electron withdrawing groups in the *para* position might increase the potency of the compound. Barbachyn *et al*. introduced a stronger electron withdrawing group (e.g. fluorine) in their test compound **27** to determine its antibacterial activity. Eventually, they proved that fluorine substitutions had significantly enhanced the potency better than that with chlorine substitution.

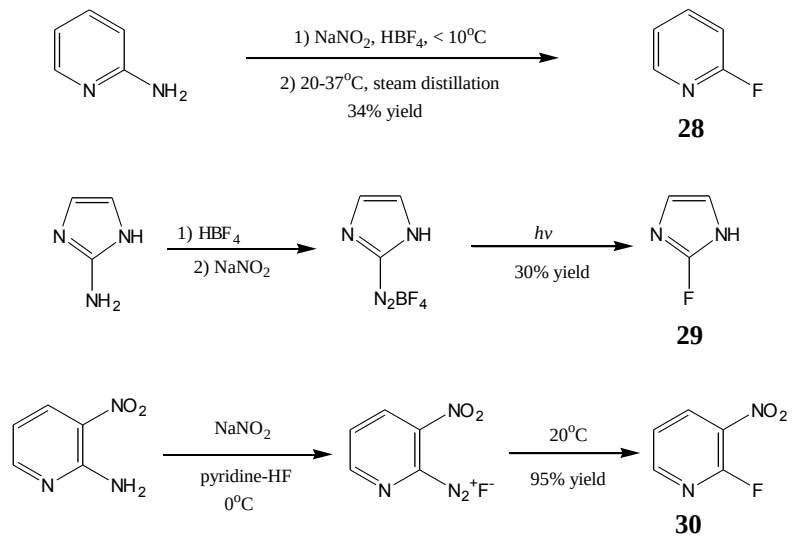


27

(R^1 = alkoxy, amino; R^2 = H, F; R^3 = F, Cl, CF_3)

1.4.1 Introduction of fluorine into heterocyclic and aromatic compounds

Many reviews have illustrated that special fluorinating reagents are commonly used to introduce elemental fluorine into heterocycles (Scheme 3)[77-81]. Further fluorination of heterocycles [compound **28** – **30**] can also be carried out by using the Balz-Schiemann reaction[82] to convert $–NH_2$ to –F, or halogen exchange methods[83], or reaction with high-valency metal fluorides[84].

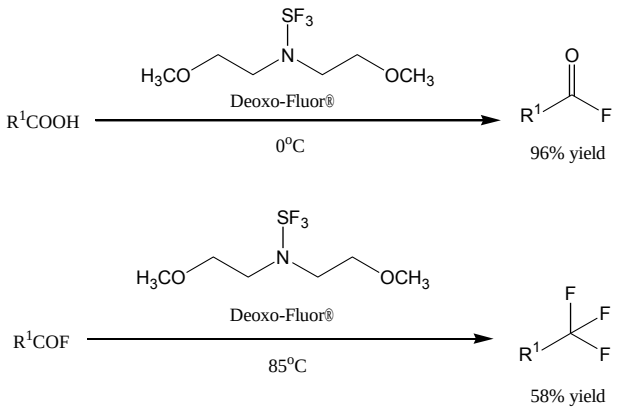


Scheme 3: Examples of the introduction of fluorine into heterocycles

Another typical example is that of the fluoroquinolones, which have been mentioned previously. Early organometallic fluorinating reagents, because of their limited thermal stability, caused the incorporation of fluorine into organic molecules to be less developed than in the case of early work with hydrocarbons[85]. Later, fluorinated organometallic reagents were developed with good thermal stability and so more fluorinated organometallic compounds were synthesized. An example of the effect of aryl ring fluorination on the antimicrobial activities was the compound **12c**[53] which showed significant enhancement of activity on Gram negative bacteria.

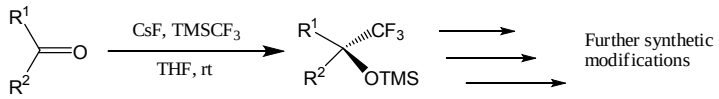
1.4.2 Trifluoromethyl substitution in organic compounds

Bis (2 – methoxyethyl) amino sulfurtrifluoride (Deoxo – Fluor®) discovered by Lal and co-workers[86, 87] is a very versatile fluorinating reagent in organic synthesis serving as a thermally stable alternative to (diethylamino)sulfurtrifluoride (DAST) and can transform carboxylic acids to acid fluorides or trifluoromethyl derivatives. (Scheme 4)



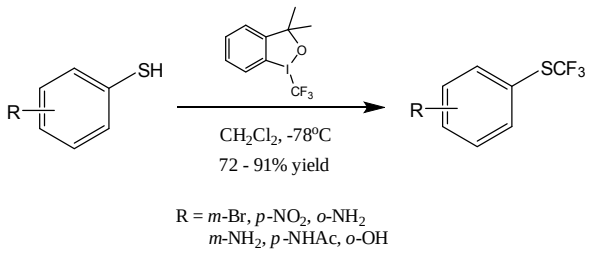
Scheme 4: Trifluoromethylation of carboxylic acids via Deoxo – Fluor®

Trimethyl(trifluoromethyl)silane, $(CH_3)_3SiCF_3$, (Ruppert – Prakash reagent) is widely used for nucleophilic trifluoromethylation. (Scheme 5)[88, 89]



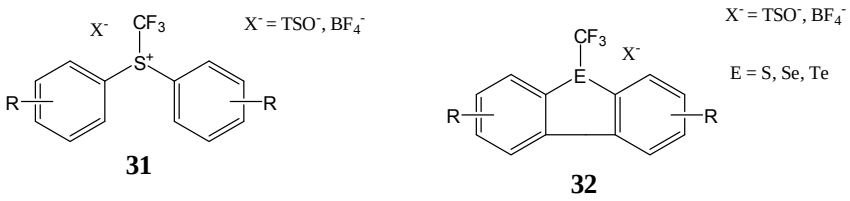
Scheme 5: Nucleophilic trifluoromethylation by means of Ruppert – Prakash reagent

1,3-Dihydro-3,3-dimethyl-1-(trifluoromethyl)-1,2-benzodioxole known as the Togni reagent is an electrophilic trifluoromethylation reagent based on hypervalent iodine. (Scheme 6)[90]

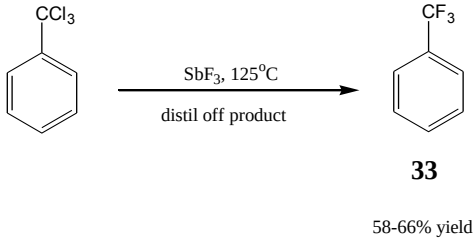


Scheme 6: Electrophilic trifluoromethylation via Togni reagent

The bis aryl thiotrifluoromethyl reagents shown below are also electrophilic trifluoromethylating reagents [compound **31**, **32**].[91]

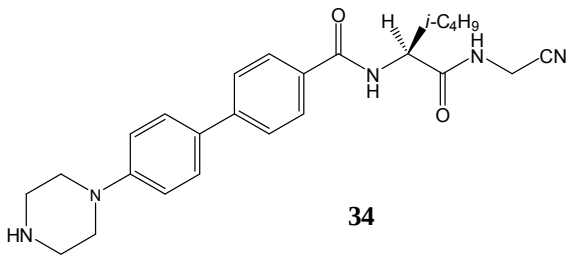


Trifluoromethylated aromatics can also be prepared by converting benzotrichloride into benzotrifluoride [compound **33**] (Scheme 7).[92] Hydrogen fluoride could also be used.[93]



Scheme 7: Conversion of benzotrichloride into benzotrifluoride

The trifluoromethyl group (-CF_3) itself has a high electron withdrawing effect similar to that of oxygen[94] and has a size slightly larger than an isopropyl group[39]. The effect of substitution of –CF_3 on organic compounds is to assist the changing of regioselectivity[95] (from an –(*S*) or –(*R*) enantiomer transformed into a chiral compound) and reactivity[96] (by producing different compounds by the same chemical reaction (compared to –CH_3) of the compounds. M^{c}Clinton and M^{c}Clinton[97] have commented that –CF_3 causes minimal disruption to an enzyme substrate complex[98] due to only slight effect of the bond length when a trifluoromethyl group replaces a methyl group attached to a carbon. As well as this, Maier *et al*.[99] and Reynolds *et al*.[100] pointed out the high lipophilicity of -CF_3 in pharmaceutical and agrochemical compounds. When present showed an improvement in membrane transport characteristics *in vivo*, thereby facilitating lower dosage. Muller[101] suggested lipophilicity is strongly dependent on the position of the fluorine within the molecule.



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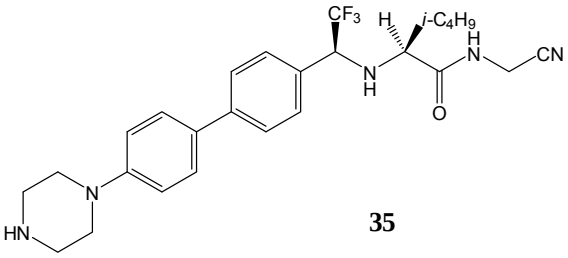
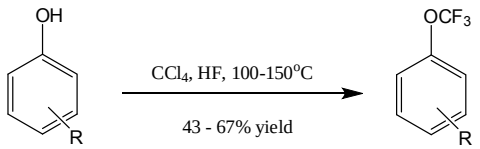


35

The trifluoromethyl containing compound 35 showed at least 3-fold potency of inhibition over the non-trifluoro substituted compound [34].[102]

1.4.3 Trifluoromethoxy substitution in organic compounds

The trifluoromethoxy group (-OCF_3), should impart physical properties consistent with its higher electron withdrawing ability and may alter lipophilicity compared with its methoxy analogue[103]. Due to its unusual stability, the -OCF_3 group is strongly resistant to strong acids, strong bases and strong oxidizing and reducing conditions[104, 105]. Trifluoromethoxy compounds [36] (or trifluoromethyl ethers) can be prepared by reacting a variety of substituted phenols with hydrogen fluoride in excess carbon tetrachloride in a closed pressure vessel under autogeneous pressure[106]. (pressure generated from the reaction) (Scheme 8)



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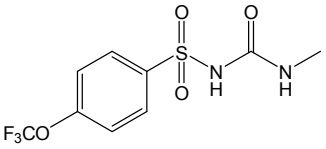
Scheme 8: Conversion of substituted phenol to trifluoromethyl ether derivative

The deactivation of the aromatic system occurs when there is substitution of the trifluoromethoxy group on an aromatic ring, even though a trifluoromethoxy group could exhibit electron withdrawing behavior similar to the halogens[107]. It has been shown that the following substituted trifluoromethoxyphenyl compound [37] has valuable pharmacological activity (as a hypoglycemic agent to lower blood glucose level)[108].



Ref. 108

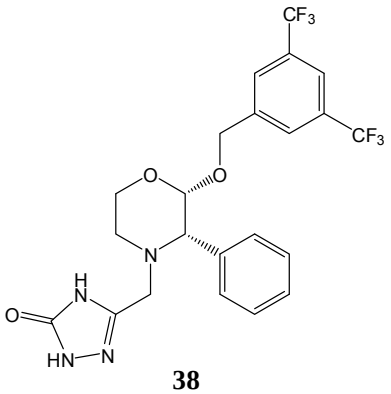
37

1.5 The roles of fluorine in medicinal chemistry

Drugs containing fluorine atoms have constituted around 5 to 15% of the total number of drugs on the world market over the past 50 years[39]. Medicinal chemists will have much more success in synthesizing and designing new fluorinated drugs now that new fluorinating methodologies and fluorinated commercial intermediates continue to be made available. Recent reviews by Kirk[44] and Hagmann[39] highlighted recent developments of fluorine containing drugs.

Some examples of fluorine in medicinal chemistry:

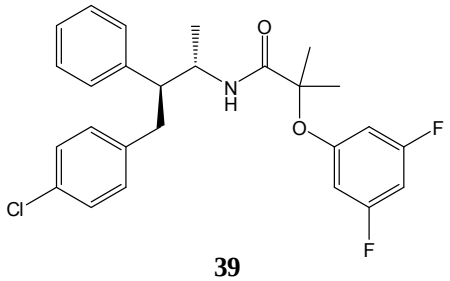
3-(((2*S*,3*S*)-2-(3,5-bis(trifluoromethyl)benzyloxy)-3-phenylmorpholino)methyl)-1*H*-1,2,4-triazol-5(4*H*)-one [compound 38][109]



38

NK-1 Antagonists, in vivo potency being improved by fluorine substitution. Removal of any $-CF_3$ group from the compound results in a 3-fold decrease in receptor affinity. This drug is for treatment of chemotherapy induced nausea and vomiting. Also NK-1 antagonists contain a bis-trifluoromethylphenyl group would help for central nervous system penetration.

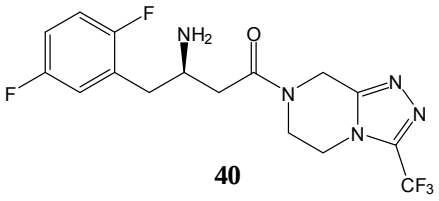
_N_-((2_S_,3_S_)-4-(4-chlorophenyl)-3-phenylbutan-2-yl)-2-(3,5-difluorophenoxy)-2-methylpropanamide [compound 39][110]



39

Covalent binding of cannabinoid-1 receptor was improved by introduction of fluorine atoms. The covalent protein binding and bioavailability were 2-fold improved by two additional of fluorine atoms on the phenoxy ring.

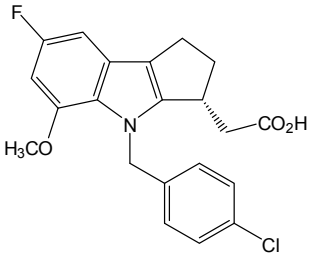
(_R_)-3-amino-4-(2,5-difluorophenyl)-1-(3-trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-_a_]pyrazin-7(8_H_)-yl)butan-1-one [compound 40][111]



40

Enhanced protency of a DPP-4. Treatment for Type 2 Diabetes, this 2,5-difluorophenyl derivative was almost 5-fold more potent than the fluorine free dipeptidyl peptidase-4 inhibitors (DPP-4). It showed that fluorine substitutions on the phenyl ring of triazolopiperazine of DPP-4 played an important role in the improvement in potency and pharmacokinetics.

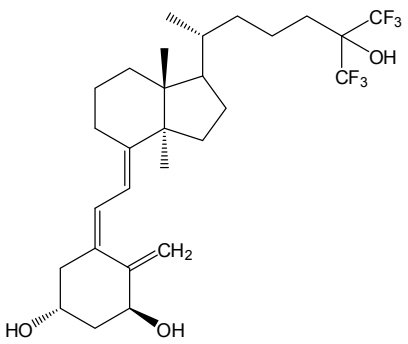
[(3R)-4-(4-Chlorobenzyl)-7-fluoro-5-acetyl-1,2,3,4-tetrahydrocyclopenta-[b]indol-3-yl]acetic Acid [compound 41][112]



41

D_2 Prostaglandin Receptor Antagonists. Treatment of allergic rhinitis, the parent compound containing methylsulfone group was replaced by a fluorine atom and improved the biliary properties, plasma clearance properties and lengthened the plasma half-life of the drug.

Falicalcitral [compound 42][113]



42

Falicalcitral an example of increased metabolic stability by fluorine substitution. This fluorinated compound is 5-fold more potent in healing rickets and elevating serum inorganic phosphorus levels of rachitic rats and in increasing intestinal calcium transport and calcium mobilization of vitamin D deficient rats. Falicalcitral is 10-fold more active than Vitamine D_3.

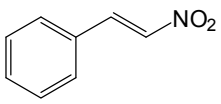
These successful biochemical outcomes became the lead compounds for later rational drug design.

1.6 Significance of the project

The increasing microbial resistance of clinically important bacteria to current antibiotics is of great concern for public health suggesting an increased need for new, more effective and safer antibacterial agents. The aim of this project is to develop highly efficient β-nitrostyrene derivatives as antibacterial agents. Previous research on the structure activity relationships (SAR) of these compounds for antibacterial activity revealed that a substance with a fluorine substituent on the benzene ring showed the highest activity against Gram negative bacteria[53]. Furthermore, the number of new effective antimicrobial agents with selective toxicity brought to the market in the past 20 years has been very low. It is necessary to synthesize and design more similar or novel compounds based on the fluorinated nitrostyrene compound [**12c**] as well as identifying their antibacterial properties.

1.7 Chemistry of β-nitrostyrenes

1.7.1 The Chemical properties of β-nitrostyrene

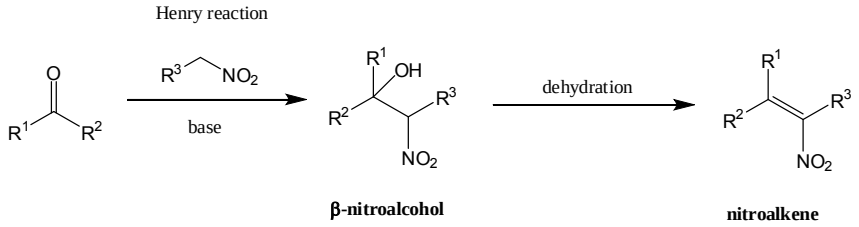


β-nitrostyrene, 1a

β-Nitrostyrene [**1a**] is the parent member of the aryl-nitroalkene family, and is a yellow crystalline solid of molecular weight 149 g/mol. It dissolves in organic solvents such as acetone, ethanol and dichloromethane, but not in water. It has a partition coefficient (octanol/water) of 59 and a melting point range of 58-59°C[114, 115].

1.7.2 The nature of nitroalkenes and their applications in chemistry

Nitroalkenes are, generally prepared by the aldol condensation between carbonyl compounds and nitroalkanes via the β-nitroalcohol intermediate (nitroaldol) and this is known as the Henry reaction (Scheme 9). This reaction has found widespread application in synthetic organic chemistry[116-123].



Applications:
Michael acceptors
Dienophiles
Domino reactions
Pyrrole formation
Friedel-Crafts alkylation
Masked ketones

Scheme 9: General synthesis of nitroalkenes

Dehydration of the β-nitroalcohol intermediate forms nitroalkenes and these compounds have found a variety of applications such as Michael acceptors[117, 124-131], dienophiles[118, 132-141], domino reactions[142], masked ketones[143-145], pyrrole formation[146, 147], Friedel-Crafts alkylation[148, 149].

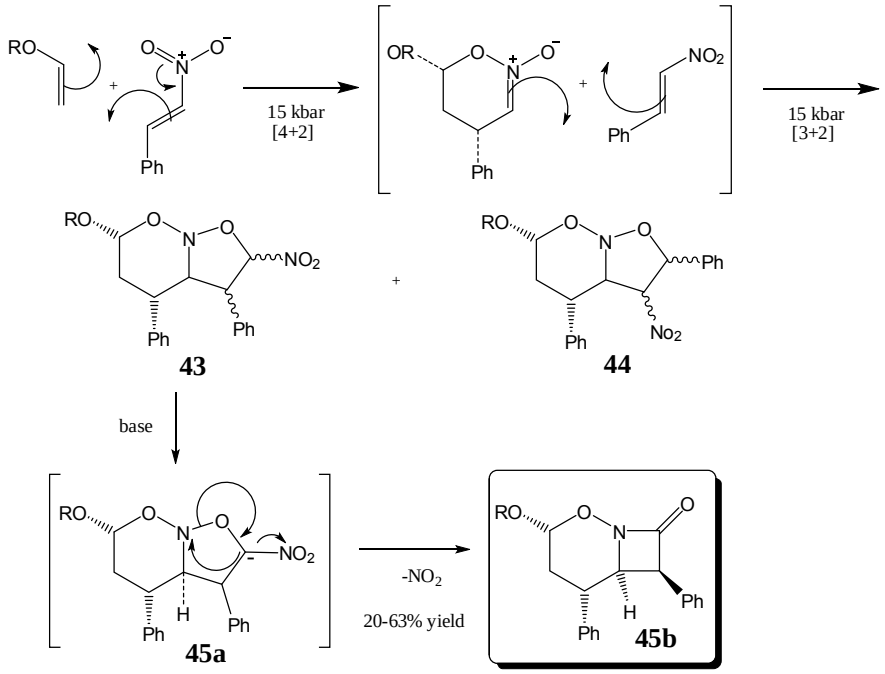
The Henry reaction is characterized by:

a) Reaction products that are usually formed as diasteriomeric syn- and anti-mixtures. The modification of the experimental conditions can result in the isolation of β-nitro alcohols with high diastereoselectivity.
b) A variety of ionic and non-ionic bases can be used including: sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium methoxide (CH_3ONa), potassium methoxide (CH_3OK), potassium tert-butoxide (*t*-BuOK), ammonium acetate (CH_3COONH_4), sodium carbonate (Na_2CO_3), potassium carbonate (K_2CO_3), potassium fluoride (KF), solid supported bases, amines, 1,5-diazabicyclo[5.4.0]undec-ene-5 (DBU) and 1,5-diazabicyclo[5.4.0]nonene-5 (DBN).
c) Only catalytic quantities of base are required and mildly basic conditions are necessary for the dehydration.
d) Typical reported yields are in the range 11 – 95%[120, 133] (depending on types of catalysts and solvents used).

1.7.3 Some synthetic applications of nitroalkenes

Diels-Alder or cycloaddition reactions

Nitroalkenes can act as powerful electron withdrawing substituents which makes nitroalkene derivatives potent dienophiles, undergoing the Diels-Alder or cycloaddition reactions[132] (Scheme 10).

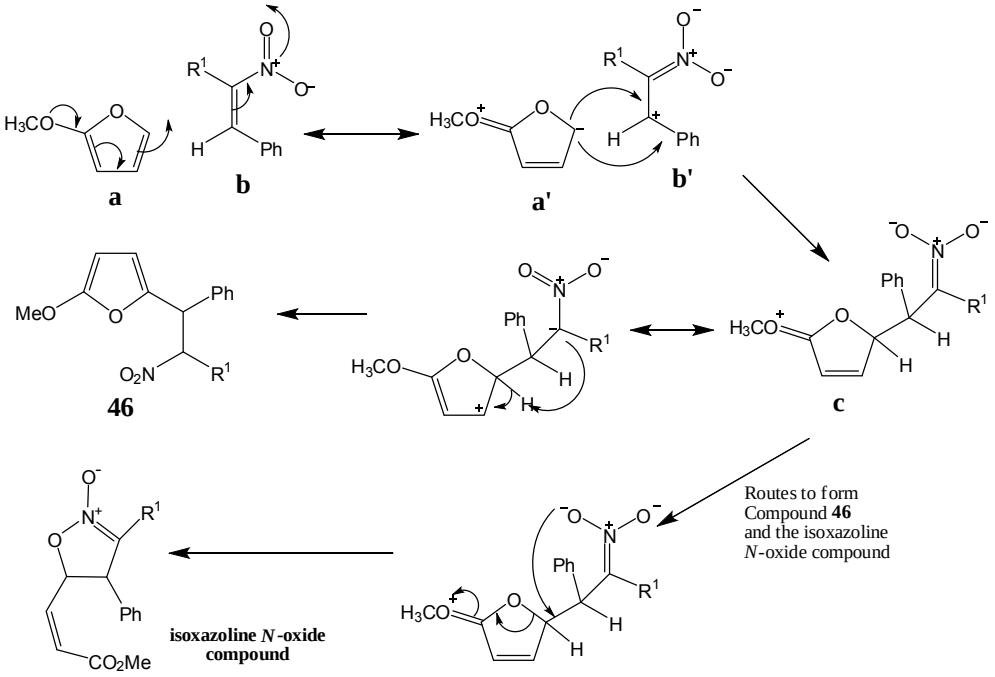


Scheme 10: Novel class of di- *N*-oxy-β-lactam compounds by cycloaddition reactions

Scheme 10 shows β-nitrostyrene has been used (4 equivalents in the reaction) to form regioisomeric products **43** and **44**. The functions of β-nitrostyrene in this reaction are that they firstly act as an electron-poor diene in an inverse electron demand in the Diels-Alder reaction with enol ether, which is an electron rich compound. After that, the electron poor intermediate **45a** (dipolarophile) reacts with another β-nitrostyrene to form 1,3-dipolar cycloaddition compounds [**43** and **44**]. Both first and second step reactions were done under high pressure (15 kbar); compound **45b**, which is the β-lactam compound, was surprisingly simple to purify from compound **43** by using silica gel chromatography (eluted by an eluent containing triethylamine).

Michael addition reactions

In addition, nitroalkenes are powerful electrophiles and form highly stable carbanions[150] that readily undergo asymmetric conjugate addition reactions with nucleophiles or radicals[134, 151]. β-Nitrostyrenes are also good acceptors in Michael addition reactions applied to some natural products[130], shown in Scheme 11

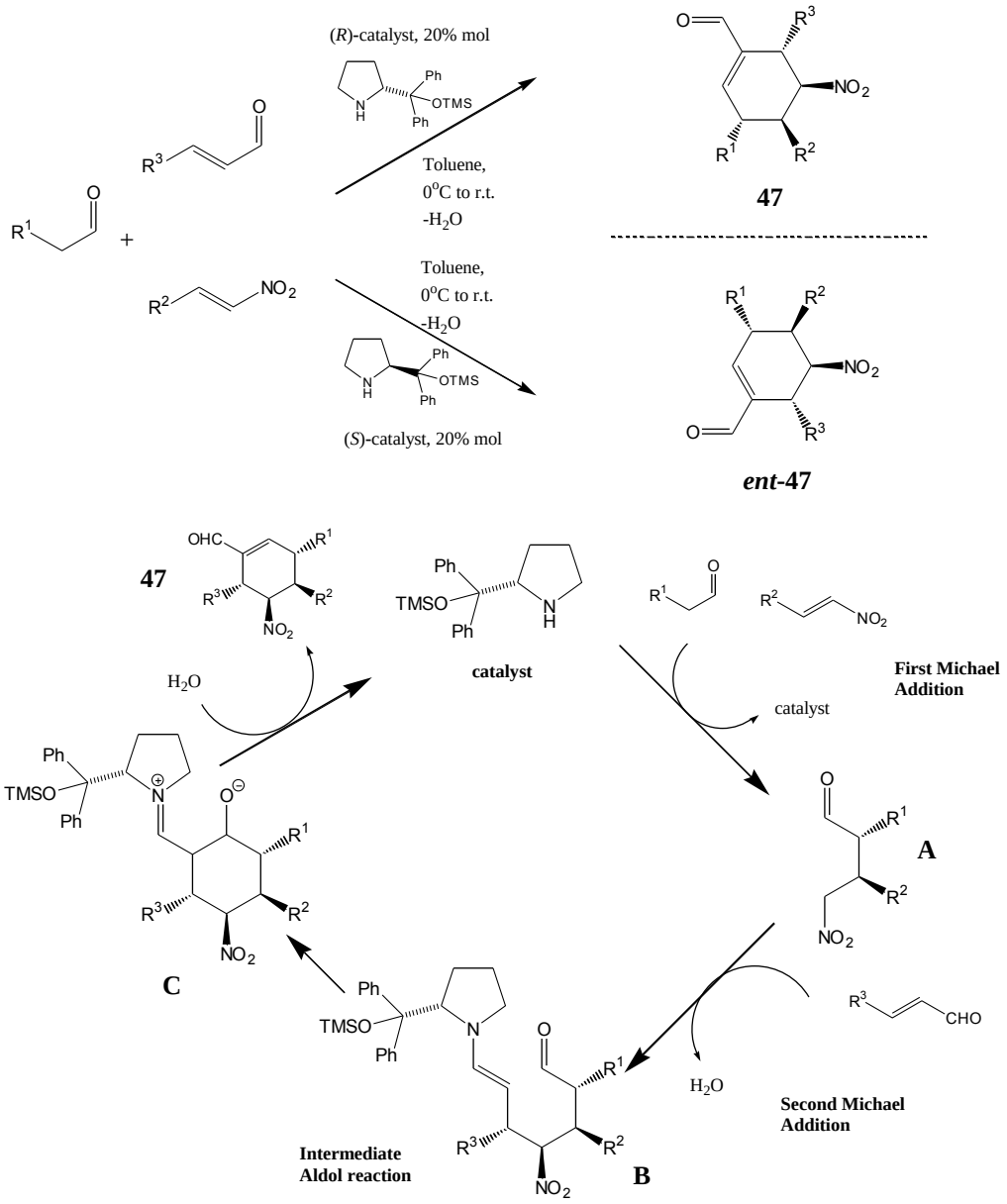


Scheme 11: Novel Formation of isoxazoline *N*-oxide with Michael adduct compound.

Whereby 11, **a′** is added to the polarized nitroalkene derivative [**b′**] to form the adduct **c**. The Michael adduct [compound **46**] could be made from **c** by electron and proton transfer. Alternatively, compound **c** could also form the isoxazoline *N*-oxide compound by ring formation. Itoh and Kishimoto[129] discovered an interesting mechanistic feature that the isoxazoline *N*-oxide ring is generated by an induction of intramolecular nucleophilic attack by the nitronate anion to the carbon-oxygen bond fission of the furan ring.

Domino Reaction

β-Nitrostyrene can be used in triple cascade organocatalytic reactions[152] (domino reactions; Scheme 12) to prepare highly substituted nitro cyclohexene derivatives.



Scheme 12: Triple cascade organocatalytic reactions

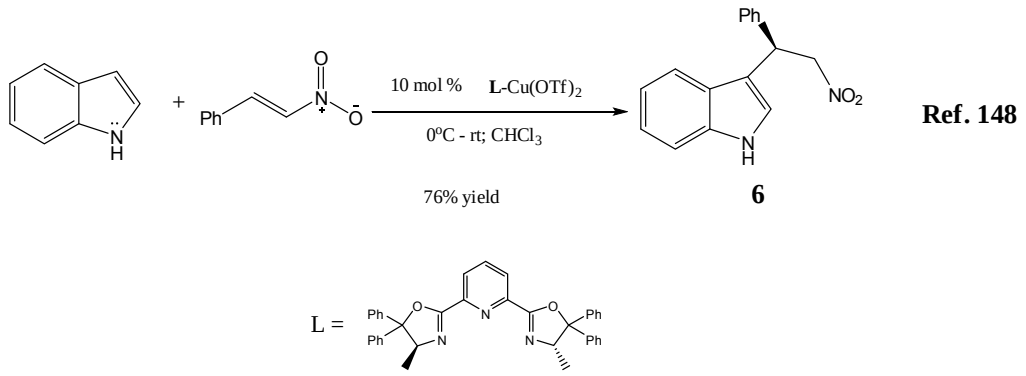
This reaction produced **47**, and ***ent*-47** with multiple stereogenic centres. The advantages of this reaction are low reaction time (16-24 hours at room temperature), and cost, including purification of intermediates and steps avoiding the protection and deprotection of functional groups. It is greener chemistry, as the reaction is environmentally friendly in that organocatalysts are used that are non toxic, the reaction is highly efficient, starting

materials are readily available and are metal-free and compounds with excellent stereoselectivities are often obtained[142].

Scheme 12 shows that the catalyst makes the enamine to be formed, which made the aldehyde to be selectively added to the nitrostyrene in Michael – type reaction. The hydrolysis process liberated the catalyst, which causing them to be able to form the iminium ion of α,β-unsaturated aldehyde to complete the conjugate addition with compound **A**. In the third step, the enamine activation of intermediate **B** makes it possible for an intramolecular aldol condensation to form compound **C**. Further hydrolysis occurred to recycle the catalyst and release the desired product compound **47**.

Friedel-Crafts alkylation

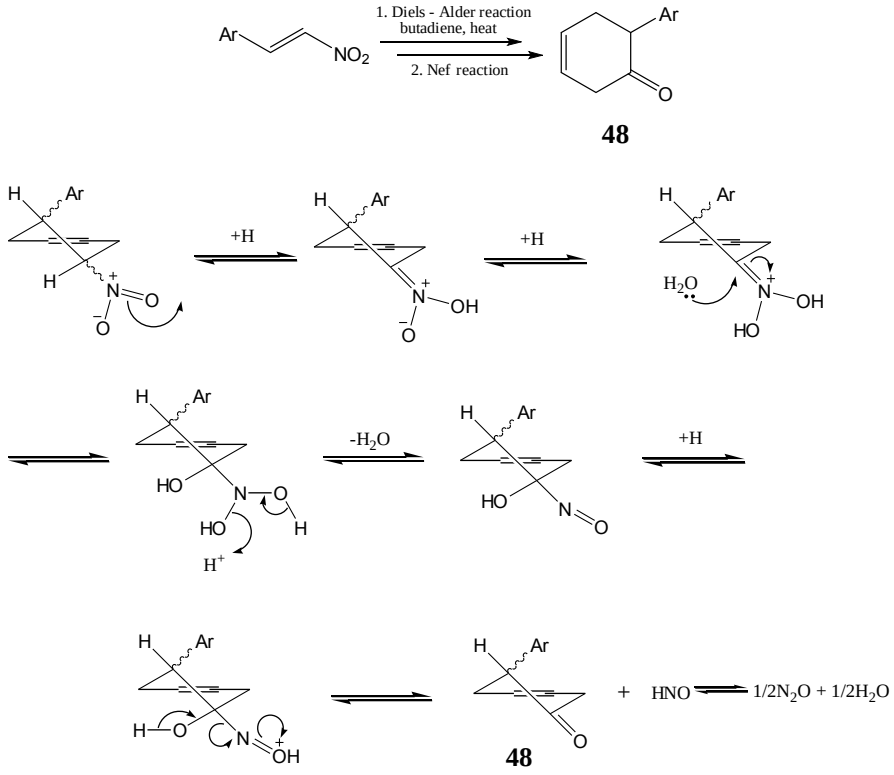
Another recent application of nitrostyrene compounds in chemistry is the enantioselective Friedel-Crafts alkylation of indoles with nitoalkenes catalyzed by different copper(II) triflate ($Cu(OTf)_2$) bisoxazoline complexes (Scheme 13)[149].



Scheme 13: Enantioselective Friedel-Crafts alkylation of indoles with *trans*- β-nitrostyrene

Nef reaction

The Nef reaction provides protocol to form masked ketone [48] compounds from nitroalkenes (Scheme 14)

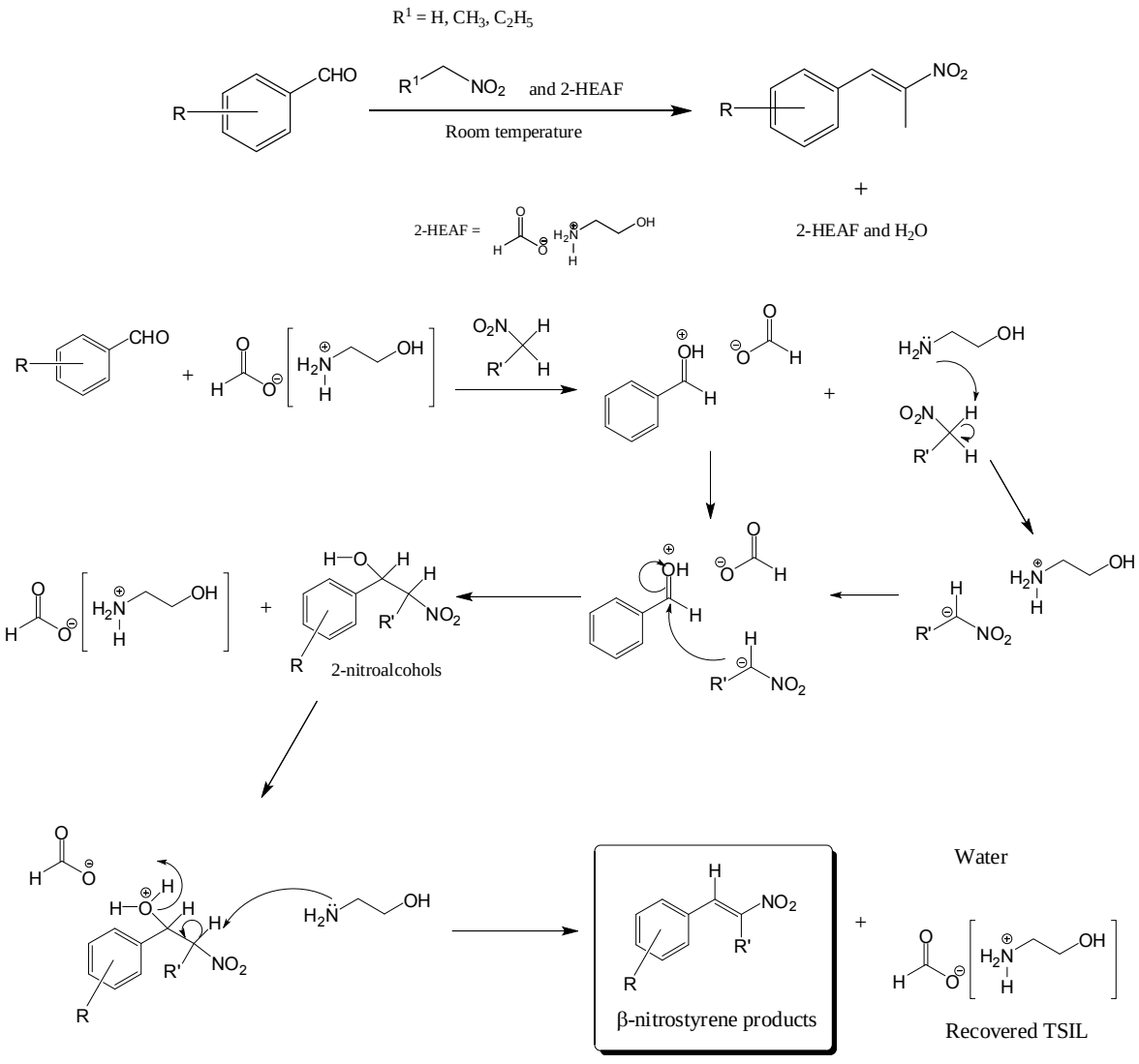


Scheme 14: Formation of ketones via Diels – Alder and Nef reactions

In summary, β-nitrostyrenes are versatile precursors for the synthesis of diverse chemical functionalities which are very reactive 1,3-dipolar reagents that can be converted into nitrile oxides, nitrones and nitronates[117-119, 124, 125, 129, 133, 134, 142, 153]. Therefore, β-nitrostyrenes (and nitroalkenes) are excellent C – C bond forming agents and are used widely in organic synthesis to prepare novel compounds.

1.7.4 Other modern methods to synthesize β-nitrostyrenes

Apart from the methods presented in Chapter 3, there are newer methods that recently have been used to make β-nitrostyrenes and a few of the methods provided a shorter reaction time, environmental friendly and excellent yield of the product. Alizadeh *et al*.[132, 154] developed a green method to synthesize β-nitrostyrenes using a cost-effective ionic liquid, 2-hydroxyethylammonium formate (2-HEAF), in the reaction. (Scheme 15)

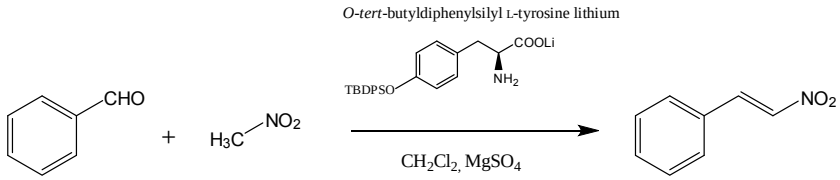


Scheme 15: A green synthesis of β-nitrostyrenes

They concluded the advantages of this reaction are this is a very clean and high yielding processing with no acid, base or metal catalyst required in the reaction. No side products were formed and all products were a crystalline forms which were easily characterized by

their melting points and spectroscopic data. As well as this one pot synthesis procedure avoided using hazardous organic volatile solvents and toxic catalyst, the reaction was done under room temperature and the use of cost-effective ionic liquids. The ionic liquids will be recovered in the procedure and can be used again. Overall commercially available and low-cost with high conductivity, great solvating ability and low melting point ionic liquids can be potentially used in other organic synthesis method.

Yoshida *et al.*[155] found the addition of dehydrating reagent, $MgSO_4$, to the reaction can improve the yield (33% up to 81%) of β-nitrostyrenes when using excessive nitroalkane (5 equivalents). Scheme 16



Scheme 16: Recent method of synthesizing β-nitrostyrenes

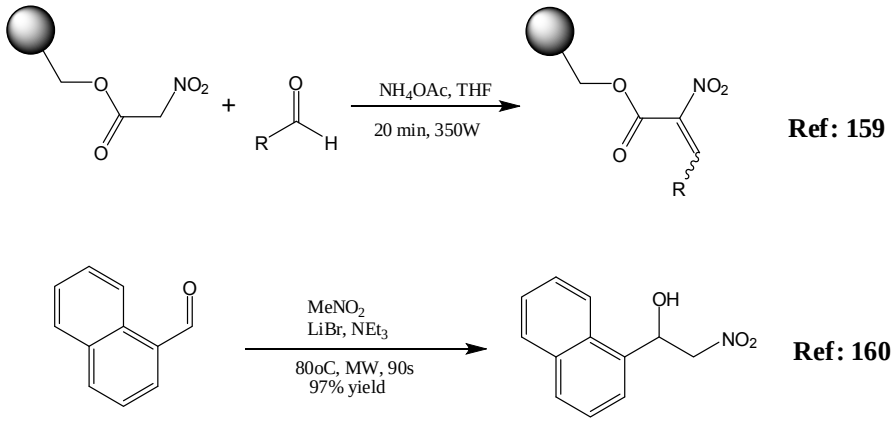
The advantages of this method are good yields obtained and only a one step synthesis of the nitroalkene. As well as this the catalyst, *O-tert*-butyldiphenylsily L-tyrosine lithium salt, can play two different roles in the reaction: it helps to form the nitroalkene, and the catalyst can be used in the next step of reaction. This can help reducing the use of catalysts, in other words cost effective. However the longer reaction time (two days) it takes to synthesize the nitroalkene and organic solvent was used in the reaction become the disadvantages of this method.

1.8 Microwave assisted Henry reactions

Microwaves were used to assist the Henry Reaction in this project. Microwave irradiation (MWI) relies on the dielectric heating properties[156-158]. All dedicated microwave reactors for chemical synthesis generally operate with frequency at 2.45 GHz[156, 159]. This thermal

effect is dependent on the polar nature of specific solvent or reagents. There are two mechanisms which make solvents absorb microwave energy and convert it into heat. The dipolar rotation mechanisms, which results from dipolar polarization as a consequence of dipole-dipole interactions between polar molecules and the electromagnetic field[158], or ionic conduction mechanisms which result when ions cluster in solution. The ions will circulate in solution by an electric field, the and collision rate will increase due to this movement in solution causing an expenditure of energy. The resulting kinetic energy is converted into heat[157]. Overall, the effectiveness of microwave irradiation is associated with the polarity of a molecule.

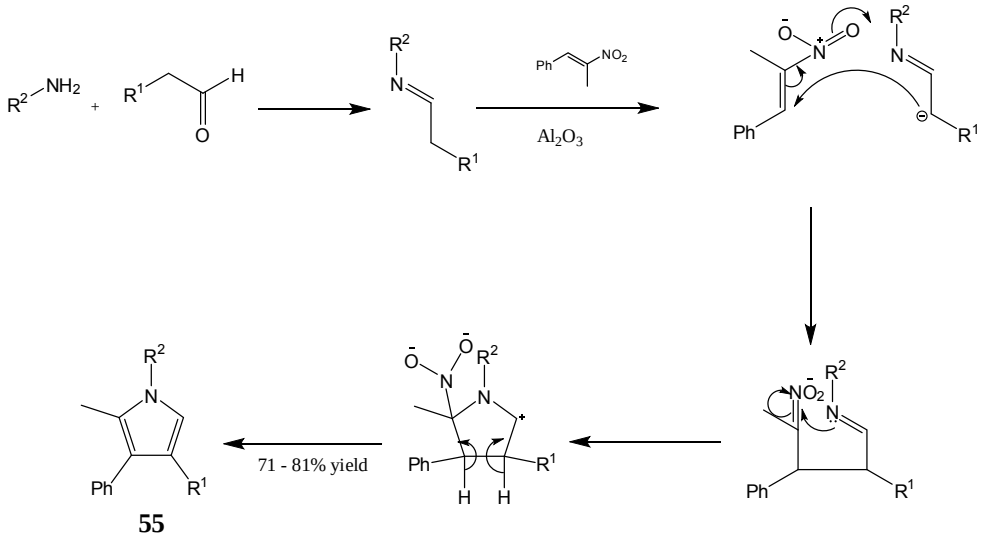
Examples of microwave assisted Henry Reactions (Scheme 17)[115, 160, 161]



Scheme 17: Microwave assisted Henry Reactions

Microwave irradiation

β-Nitrostyrene reacts with a carbonyl compound and an amine on alumina with microwave irradiation which is an efficient method of forming pyrrole compounds (Scheme 18)[147].



Scheme 18: Formation and reaction of β-methyl-β-nitrostyrene using microwave irradiation

1.9 Partition coefficients

It is important to gain some idea of the permeability of a drug to pass through living cellular membranes. For this purpose, the partition coefficients (K_D) of drugs need to be determined. Partition coefficients are used to determine the lipophilic nature of a compound. Solubility, permeability, oral absorption, cell uptake, blood-brain barrier penetration and metabolism of a compound are influenced by its degree of lipophilicity[162]. There are five key techniques for the determination of lipophilicity: solvent/water partitioning, chromatographic approaches, artificial membranes, electrokinetic approaches and partitioning between lipid/water phases[162]. A widely used technique to define the lipophilicity of a drug is the octanol/water partitioning[163-165] and this technique was used to determine the lipophilicity of each nitrostyrene derivative prepared in this work.

Chapter 2

2 Results and Discussion

2.1 Introduction

This chapter contains discussion of the synthesis and biological activity of nitroarenes, with emphasis on substituted 2-nitroprop-1-enyl benzenes. The antibacterial efficacy of the products was assessed by their activity against a panel of bacteria and a fungus (*Candida albicans*). The results appear as the minimum inhibitory concentration (MIC) for each compound. The partition coefficient (K_D) between octanol and water for each compound, representing its degree of lipophilicity, was also determined in order to access the extent of its interaction with the surface of the microorganism. Compounds with the incorporation of fluorine were of considerable interest in these studies, particularly as earlier studies had indicated an improvement of antibacterial activity from this approach. Results are discussed in terms of structure-activity relationships that are important for activity against the microorganisms studied.

2.2 Synthesis of 2-nitroprop-1-enyl benzene derivatives

The Henry reaction was utilized for the preparation of most of the compounds. Two different conditions, referred to as Method A, Method B and other variations from the literature were used.

Method A used methylamine as catalyst and was carried out at room temperature under mild alkaline conditions using sodium carbonate. Method B was performed in glacial acetic acid with ammonium acetate at 100°C or higher (see details in *Chapter 3*). In some cases Method A gave better yields than Method B, while in other cases, the reverse applied. Generally, the NMR data and mass spectrum of each compound were sufficient

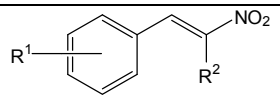
as a guide for purity especially if the final melting point after recrystallization was sharp (range 1 - 2℃).

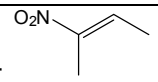
2.3 The Henry reaction

The Henry Reaction (refer to *Chapter 1*) can be used for the preparation of nitroalkenes and this reaction was the dominant reaction used in this project. The condensation was performed under various conditions. One technique was based on the method of Knoevenagel and Walter[166]. In this condensation reaction, the aldehyde and nitro compound react in the presence of potassium carbonate and methylamine in a solvent (ethanol) at room temperature. According to Crowell and Ramirez[167], and Crowell and Kim[168] the key reagent for this reaction is the amine, which acts as a catalyst in the reaction. The amine catalyst reacts with benzaldehyde to form an imine and water as a by-product. The water produced in the reaction, in fact, was found to have an appreciable effect on the yield, as it will shift the equilibrium of the reaction to the left, hence reducing the yield of the product[167, 168]. For this reason ethanol was introduced to reduce the influence of water and so optimize the yield[168].

A table below (Table 3) showed the structure, yield and melting points of compounds that have been synthezied in this research project. Synthesis methods and conditions can refer to *Chapter 3* the experimental section.

Table 3: Synthesized compounds.



Compound	R1	R2	Yield (%)	M.P. (°C)
1a	H	H	82	58-59
1b	4-F	H	57	99-100
8b	4-OH	CH_3	50	121-122
9b	3,4-dimethoxy	CH_3	27	71-72
11	H	CH_3	22	60-62
12a	2-F	CH_3	34	45-47
12b	3-F	CH_3	52	NA
12C	4-F	CH_3	30	65-66
12d	2,4-difluoro	CH_3	43	48-49
49a	2-CF_3	CH_3	46	NA
49b	3-CF_3	CH_3	46	NA
49c	4-CF_3	CH_3	42	96-98
50a	3-OCF_3	CH_3	51	NA
50b	4-OCF_3	CH_3	73	47-48
51	3- 	CH_3	18	99-101
52	4- CH_3	CH_3	80	54-55
53a	1-naphth	CH_3	49	62-64
53b	2-naphth	CH_3	41	90-91
54a	H	phenyl-benzpyran	43	88-90
54b	H	4’ fluorophenyl-benzpyran	55	88-89
55	H	CH_2CH_3	60	NA

2.4 Structure-activity relationships (SARs)

The important structural features studied were

1. An aromatic ring (most compounds were based on β-nitrostyrene)
2. A nitro group on alkenyl side chain
3. Other substituents on the aromatic ring
4. Variations in the structure of the side chain

Most compounds tested for microbiological activity possessed an aromatic ring. Previous studies likewise have been carried out on aromatic compounds as it was thought that the flat surface of the aromatic ring could facilitate van da Waals bonding to other flat structures in the microorganism or other molecules that interfere with metabolism within the cell, such as enzyme inhibitors. Likewise, the nitro group was treated as a fundamental group for Investigation. The only variation being that one compound [**51**] (page 71) possessed two nitro groups. The side chain to which the nitro group was attached was unsaturated and was varied from two to four carbon atoms The compound with three carbon atoms (propenyl group) has been shown in previous studies[22] to give superior activity to those with two carbon atoms.

Other aromatic ring substituents included: –OH, $-OCH_3$, $-O-CH_2-O-$, $-CH_3$, -F, $-CF_3$, $-OCF_3$, including multiple substituents or otherwise varied according to the position on the ring. Two unsubstituted compounds [**1a** and **11**] were included as controls for these substitution effects.

2.5 The importance of previous work

The previous experimental results by Nicoletti *et al*. (unpublished work)[29] influenced the direction of this project. Comparisons were made of activity against the bacteria and the fungus common to both studies. The Nicoletti *et al* studies showed that:

a) *E. coli* (Gram negative bacterium) was suppressed effectively by chloro or fluoro substituents at the 4-position relative to the side chain of β-methyl-β-nitrostyrene; β-methyl-β-nitrostyrene with a methylene-dioxy ring substitution at positions 3- and 4- on the aromatic ring was not as effective.

b) *S. aureus* (Gram positive bacterium) was suppressed effectively by a wide range of nitropropenyl arenes including β-methyl-β-nitrostyrene, the 4-fluoro and 4-chloro substituted derivatives of β-methyl-β-nitrostyrene. Imidazolyl, 3,4-dihydroxy and benzothiazole derivatives and the 3,4-methylene dioxy derivative were also very active. An important finding was that with two hydroxy groups in the 2- and 4-positions or the 2- or 5- positions, activity against this microorganism was noticeably reduced. The fact that this also occurred with substitution by *N*,*N*-dimethyl and *N*,*N*-diethyl groups indicated that the more polar nature of these derivatives was detrimental to activity. This was supported by the K_D values of the latter compounds being relatively low compared with the unsubstituted and halogenated-substituted compounds.

c) *B. subtilis* (Gram positive bacterium) was suppressed by a wide range of compounds in a similar way to *S. aureus* and the dihydroxy substituted compounds [2,4- and 2,5-isomers] derivatives were unsatisfactory as chemical agents against this bacterium. However, 3,4-dihydroxy substitution gave high activity.

d) *C. albicans* was suppressed by the 4-chloro and 4-fluoro derivatives, as well as the 3,4-dichloro derivative. However 4-fluoro and the benzothiazole derivatives were also very active as well as 3,4-dihydroxy substituted compound.

Previous results indicated that 3,4-methylenedioxy-β-methyl-β-nitrostyrene and many of the aromatic nitro compounds were not very effective against *E. coli*. The methylene dioxy group was not quite as effective as a simple –OH at position 3 relative to the side chain (3-hydroxy-β-methyl-β-nitrostyrene) or dihydroxy (positions 3 and 4 to the side chain). The 4-fluoro substituent was superior to all other substitutions and also to the β-methyl-β-

nitrostyrene. No improvement in activity was favored by the use of a combination of –OH and $–OCH_3$ (3-hydroxy-4-methoxy-β-methyl-β-nitrostyrene and 2-methoxy-3-hydroxy-β-methyl-β-nitrostyrene) or by 3,4-dimethoxy groups, although not all possible substituted positions were tested. These results suggested that compounds having some degree of hydrophilicity were the most effective against *E. coli* and this is borne out by the relatively low K_D values of the most effective compounds (K_D 65 – 150) compared with ineffective ones such as 3,4-methylenedioxy-β-methyl-β-nitrostyrene (K_D 362). These compounds correspond to $\log_{10} K_D$ values of 1.8 – 2.2, is often referred to as optimal Log *P* values for antibacterial activity.[22] It could be speculated that for many Gram negative bacteria (such as *E. coli*) which are known to have polysaccharide structures, there would be greater affinity for hydrophilic compounds and hence passage through cell walls of these types of bacteria would be facilitated. For our results, the only Log *P* value for that can be cited for an effective fluorinated compound on *E. coli* (Gram negative) is 2.00 [**12c**]. For the Gram positive bacteria, a range of Log *P* values of 1.15 – 2.19 appeared to be related to efficacy.

For the non-fluorinated compounds, optimal Log *P* values were over a much wider range from 1.61 – 3.41, and if *C. albicans* is included the range is even wider, from 1.15 – 3.41.

2.6 Initial experiments

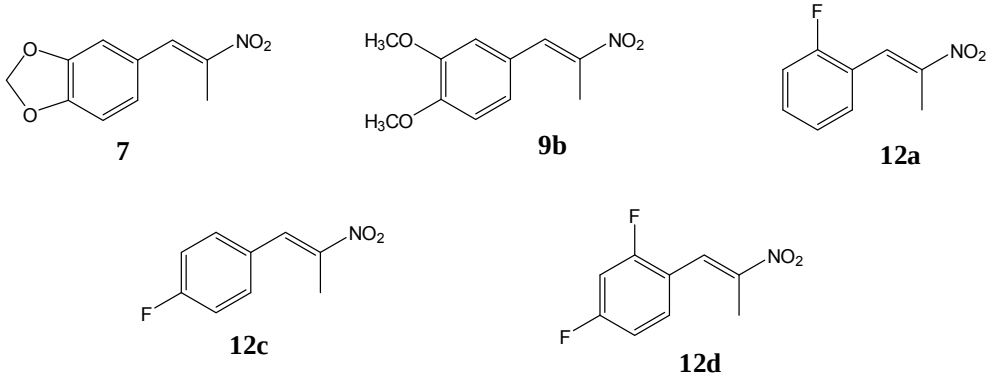
The initial experiments were performed on key compounds that would be expected to provide acceptable standards for high activity. In this respect, 4-fluoro-β-methyl-β-nitrostyrene was chosen as the most promising against *E. coli* and was compared against the 2-fluoro and 2,4-difluoro compounds. The 3,4-dimethoxy derivative of β-methyl-β-nitrostyrene was also compared against the 3,4-methylene-dioxy compound. The unsubstituted compound was also tested in the main series of experiments. It was important to show that Method A (base catalysed reaction, which usually been used by Professor Hugh Cornell in this project) produced compounds of equal activity to those

prepared by Method B (ammonium acetate – acetic acid). Finally, the wide range of partition coefficients (K_D values) of the compounds tested was wide (65 - 362) and therefore ideal for testing correlations with MIC values for both Gram positive and Gram negative bacteria. The results of these experiments are shown in Table 4, which lists the MIC values for each compound and their K_D values. Three Gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecalis*), one Gram negative bacterium (*Escherichia coli*) and a fungus (*Candida albicans*) were tested in this program.

Table 4: Geometric mean MIC values in µg/mL and K_D values for initial compounds tested against a fungus and a panel of bacteria.

Strain	Compounds and MIC values (µg/mL)				
	7	**9b**	**12a**	**12c**	**12d**
S. aureus	2	2	8	2	4
B. subtilis	2	2	3	4	2
E. faecalis	4	5	5	5.5	6
E. coli	256	128	42	27	45
C. albicans	3	4	3	2	3
Partition Coefficient (K_D)	362	250	138	101	65

Figure 1: The compounds tested for antibacterial activity



7: 3,4-methylenedioxy-β-methyl-β-nitrostyrene

9b: 2-dimethoxy-4-(2-nitroprop-1-enyl)benzene (3,4-dimethoxy-β-methyl-β-nitrostyrene)

12a: 1-fluoro-2-(2-nitroprop-1-enyl)benzene (2-fluoro-β-methyl-β-nitrostyrene)

12c: 1-fluoro-4-(2-nitroprop-1-enyl)benzene (4-fluoro-β-methyl-β-nitrostyrene)

12d: 1,3-difluoro-4-(2-nitroprop-1-enyl)benzene (2,4-difluro-β-methyl-β-nitrostyrene)

The results in Table 3 were tested for correlation between MIC value and K_D value for *E. coli* and *E.facaelis*.

Figure 2: Correlation between MIC value and K_D value for *E. coli*

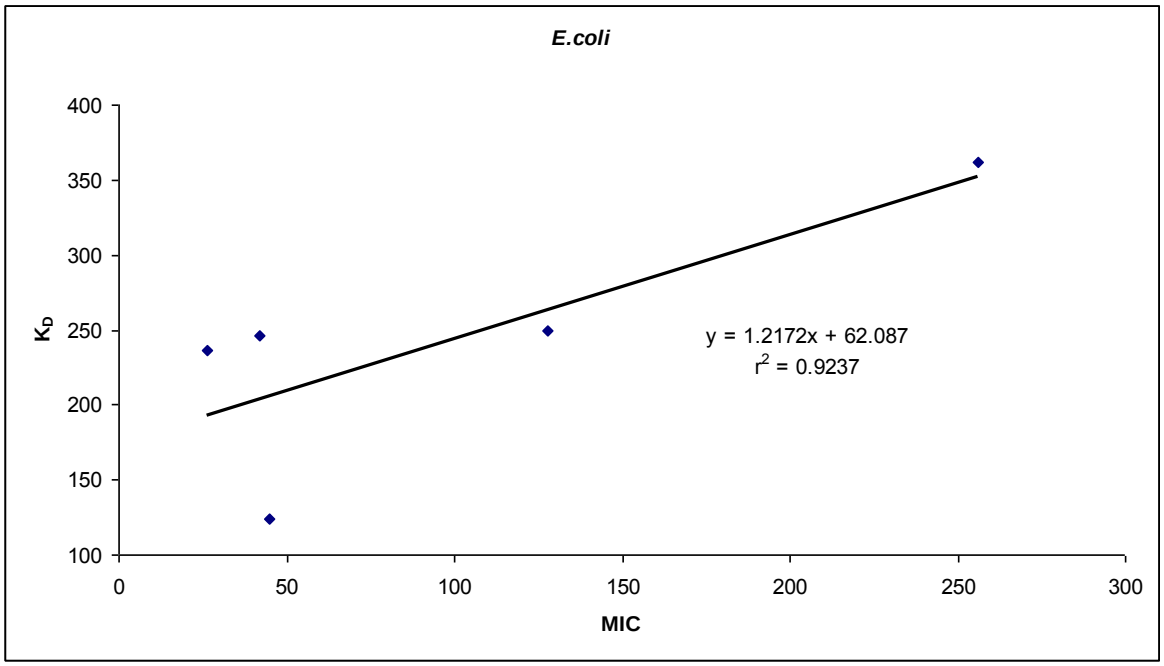
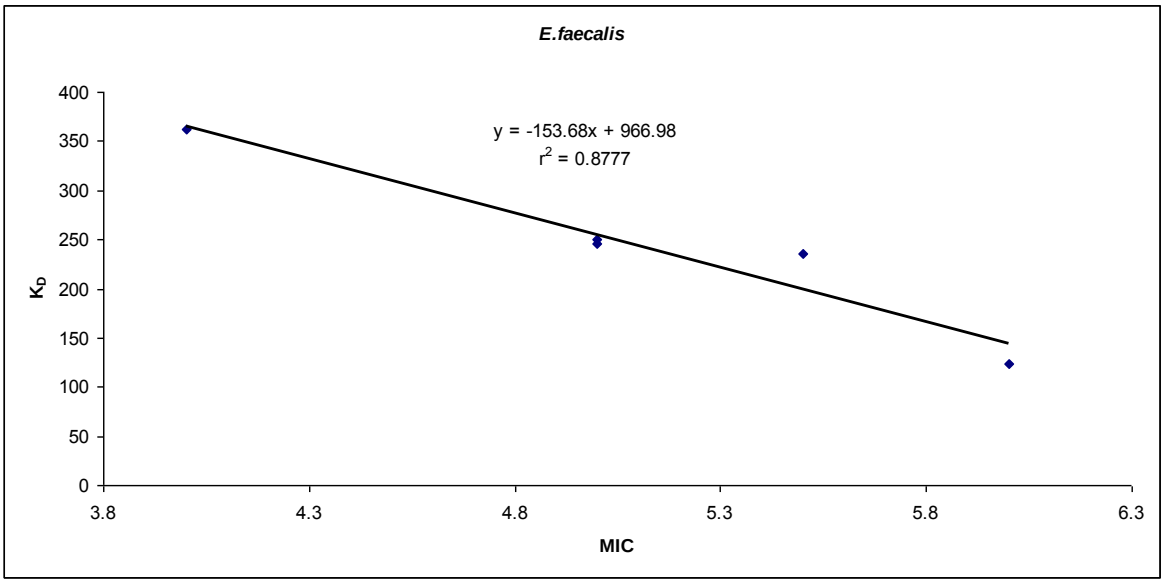


Figure 3: Correlation between MIC value and K_D value for *E. faecalis*



The results suggest that K_D values in the lowest range (65 – 138) are associated with high activity against *E. coli* whilst the opposite is the case for *E.facaelis*. Note the opposite gradients for each bacterium shown in Figures 4 and 5.

There were no correlations between MIC value and K_D value for *S. aureus*, *B. subtilis*, and *C. albicans* (graphs not shown)

2.6.1 Effects of substituents

Compound 12c

Of the five different compounds tested for antimicrobial activity, the most active compound was the one prepared from 4-fluorobenzaldehyde and nitroethane [1-fluoro-4-(2-nitroprop-1-enyl)benzene] [**12c**]. This confirmed the results obtained by Nicoletti *et al*.[29] using a panel of Gram positive bacteria, enteric and non-enteric Gram negative bacteria and fungi, in which a large number of compounds with different types of substitution on the aromatic ring were tested. There was no significant difference in activity between the two samples of the above compound, one prepared by Method A, the other by Method B. The lowest MIC values against *E. coli* (27µg/mL) were obtained with **12c** (Refer to Table 4). MIC values against the other microorganisms were all less than 8µg/mL, hence all the compounds tested would be described as very effective against the three Gram positive bacteria and *C. albicans*.

Compound 7

With regard to the *E. coli* results, the least effective compound was compound **7**. The compounds prepared from reaction of 2-fluorobenzaldehyde [**12a**] and 2,4-difluorobenzaldehyde [**12d**] with nitroethane had slightly lower activity against *E. coli* than **12c**. The compound [**9b**] prepared from reaction of 3,4-dimethoxybenzaldehyde with nitroethane gave excellent results against all microorganisms except *E. coli*. However, the latter preparation gave results with *E. coli* that were comparable to commercial compound **7**. Nicoletti *et al*.(unplublished work)[29] also evaluated some aromatic nitropropene compounds with –OH and $–OCH_3$ substitution and found that those with one –OH and one $-OCH_3$ were the most promising. The compound with dimethoxy substitution [**9b**] had high activity against the three Gram positive bacteria and the fungus common to both

experiments but was inferior to **12c** against the gram negative *E. coli*. It did, however, show relative higher activity than compound **7** (Table 4).

There was a good correlation (Figure 2) between the effectiveness against *E. coli* and the partition coefficients of the compounds tested (r^2 = 0.9237). Thus for high effectiveness against *E. coli*, compounds with low K_D values are indicated to be the most effective, i.e. compounds with low lipophilicity. In Figure 3, there is also a good correlation (r^2 = 0.8777) between the effectiveness against *E. faecalis* and the partition coefficients of the compound tested. The results show that for high effectiveness against *E. faecalis*, compounds with high K_D values are the most effective, i.e. compounds with high lipophilicity. *E. faecalis* is an enteric bacterium and its cell wall structure governs penetration of antibacterial compounds. High K_D values appeared to be favoured for high activity. No conclusions could be drawn concerning correlations with other Gram positive bacteria as the MIC values were all in the range 2-3. Likewise MIC values for *C. albican*s were also very low. Milhazes *et al*.[22] used *E. coli*, *E. faecalis* and *S. aureus* for test on various nitrostyrene derivatives, but found no correlation between MIC values and lipophilicity on *E. faecalis* and *S. aureus*.

The reason for the effectiveness of fluorine substitution on the aromatic ring is probably connected with the high electronegativity of fluorine, although size factors could also be important. Perhaps the electronegativity of fluorine could affect binding affinity to the binding site of the bacteria, thus causing inhibition of the enzyme.

The results indicate that further experiments with fluorine substitution would be valuable as a way of determining the structural features required for the optimal anti-bacterial activity of nitro compounds of this type. Fluorine substitutions made on both the ring and side chain will be attempted in order to present a clearer picture of structure-activity relationships and possible mechanisms of action.

2.7 Discussion of structure – activity relationships (SARs)

This section will focus on the effectiveness against the chosen bacteria and fungi of the main series of compounds investigated. For chemical structures of compounds refer to Table 5 page 69 and 70.

2.7.1 Hydroxy and methoxy substituted compounds

See **8a**, **8b**, **8c**, **9a**, **9b**, **10a**, **10b** against **11** (no substitution) and refer to Table 5

1. One substituent –OH group

   One –OH in 3-position [**8a**] to the side chain made marginal changes to the activity compare to the unsubstituted compound [**11**] and a small increase in K_D values (113 to 145) was not consistent with the slightly better result against *E. coli* caused by the substitution. The compound with substitution on the ring in 4-position [**8b**] with slightly higher in K_D values than **8a** showed better results against tested bacteria except result against *E. coli* which both of them had obtained the same MIC values (64).

2. Two –OH groups

   Two –OH groups, at position 3 and 4 relative to the side chain [**8c**] made a small improvement with the Gram positive bacteria relative to **8a**, but there was no change in activity against *E. coli*, the latter observation being consistent with virtually the same K_D value. The result with *C. albicans* was excellent (MIC 4).

3. –OH and $–OCH_3$ together

   10a with –OH at position 3 and $–OCH_3$ at position 4 to the side chain failed against *C. albicans*, as did **10b** with these positions reversed. This suggested that, as both of these compounds were reduced in effectiveness against *C. albicans* compared with the unsubstituted compound [**11**], high polarity effects of these groups reduced the activity against this fungal material. Both still maintained reasonably high activity against the Gram positive bacteria but the activity of **10a** against *E.*

coli was somewhat lower than **10b**, the latter being about the same as the unsubstituted compound.

4. One substituent $–OCH_3$ group

   A substitution of a $–OCH_3$ group in 4-position [**9a**] relative the side chain also changes the activity compared to the unsubstituted compound [**11**] giving a much more active compound against the chosen bacteria (same results obtained as compound **8b**) although a huge increase in K_D values (113 to 479) had occurred. These results, suggest that substitution in 4-position of the ring could optimize the activity of the parent compound [**11**] against the Gram positive, Gram negative bacteria and the fungus.

5. Two $–OCH_3$ groups

   Compound **9b** with $–OCH_3$ groups at positions 3 and 4 to the side chain gave excellent results on *C. albicans* and the Gram positive bacteria, in complete contrast to **10a** and **10b** with adjacent –OH and $–OCH_3$ groups. It could be argued that this was due to optimal polarity effects, but it does not explain why compound **8c** with its two hydroxy groups is also of excellent activity against *C. albicans* whereas the compounds with one of each type of substituent [**10a** and **10b**] are much inferior.

Activity of **9b** against *E. coli* was slightly less than **10b** and similar to that of **10a**.

For these –OH and $–OCH_3$ substitutions it appears that the main achievements were improved activity against Gram positive bacteria and *Candida albicans*, seen with substitution at position 3 and 4 with –OH groups [**8c**] and $–OCH_3$ groups [**9b**]. However, it must be pointed out that most of these substitutions only marginally improved the activity and in two cases [**10a** and **10b**] with one of each group as a substituent, there was a large decrease in activity against *C. albicans*. Milhazes *et al*.[22] found a dihydroxy derivative to be the most active against Gram negative bacteria, which agrees with the results obtained on *E. coli*.

Several other compounds also showed high antibacterial activity, particularly compounds **8b** or **9a**, which compared favourably with compounds with –OH and $–OCH_3$, substitutions previously tested against *E. coli*. Compounds **8a** and **8c** (Nicoletti *et al.*[29]) were more active than **7** and are marginally better than the non-substituted compound **11** and compound **10b** [1-hydroxy-2-methoxy-4-(2-nitroprop-1-enyl)benzene]. These results indicate that one –OH group gave some enhancement of activity against *E. coli*, (e.g. compound **8a**) as well as compound **8c** (with two OH groups on adjacent carbon atoms). However, compounds **10a** and **10b** each with one hydroxy group and one methoxy group were not as active and compound **9b** with adjacent methoxy groups was also less active.

With compound **8b** (4-hydroxy), two comparisons can be made against **9a** (4-methoxy) with $–OCH_3$ instead of –OH, and against compound **11**, which has no ring substitutions. The activities of **9a** and **8b** are almost identical yet **9a** has much higher K_D than **8b** (479 against 150). All results except those with *E. coli* are excellent.

Results were generally better with compound **8b** (4-hydroxy) than for Compound **11** against the chosen Gram positive bacteria, but were the same for *E. coli* and *C. albicans*, suggesting the substitution of –OH [**8b**] and $–OCH_3$ [**9a**] had improved the potency against Gram positive bacteria. K_D values of 479 [**9a**], 150 [**8b**] and 113 [**11**], suggested that a more lipophilic nature is tolerated for antibacterial properties in the case of Gram positive bacteria.

The compounds **8a** (3-hydroxy), **8c** (dihydroxy) **10a** and **10b** (Table 5) are included to offer further comparisons with the work of Nicoletti *et al*.[29] They have similar good activity against the Gram positive bacteria, but the activity of Compounds **10a** and **8c** is inferior to the others against *E. coli* and **10a** and **10b** have the lowest activity of all compounds in Table 5 against the fungus. The reasons are possibly related to the presence of the polar hydroxy group in **8a**, **10a** and **10b**. Compound **8c**, with two methoxy groups, is the best of

this series of compounds. Referring again to Table 4, it is seen that Compound **9b** (3,4-dimethoxy substitution) is very active against Gram positive bacteria, but lacks activity against *E. coli*.

2.7.2 Fluorine substitution on the ring

The effects of different fluorine substitutions on the aromatic ring on biological activity were explored further in order to determine which groups enhanced the activity of β-methyl-β-nitrostyrene [**11**], which was the main parent compound investigated. Previous results of MIC determinations on twenty different selected compounds, showed that 4-fluoro-β-methyl-β-nitrostyrene had the highest antimicrobial activity across the range of microorganisms tested (Nicoletti *et al*.[29]). It was pointed out that tests were performed using a large panel of Gram positive bacteria, Gram negative bacteria and fungi. In the present series of tests, a smaller panel of bacteria and a fungus were used to test the activities of compounds (see Fig 4). The results are shown in Table 5, the most active compounds against *E. coli* were compounds **8a**, **8b**, **8c**, **9a** and **12c**; all with MIC values of 64 µg/mL. Results with compound **11** (no substitution) indicated that the basic structure of β-methyl-β-nitrostyrene already has slightly lower activity against this bacterium compare to compounds **8** (**a**, **b**, and **c**) **9a** and **12c**. Substitution on the ring with $–OCH_3$, -OH, $-CF_3$ and -F only marginally improved this activity. However $–OCF_3$ substitution has caused a large reduction in activity, this maybe due to an opposing electronic effect of the oxygen atom, as this effect was not noticed with $–CF_3$. Results against the Gram positive bacteria and the fungus *Candida albicans* were generally good to excellent. More detailed analysis and comparisons follow.

The results again showed that the halogenated derivatives had enhanced potency, with **12c** having lower MIC values than **7**, the difference being seen clearly with the Gram negative bacterium *E. coli*. The early results (Table 4) again indicated that there was a

relationship between the MIC value and the K_D value for the compounds studied (r^2 = 0.5066, graph not shown). The interpretation of this relationship is that the low K_D value, representing a lower degree of lipophilic character, is necessary for disruption of the polysaccharide-rich membrane of the membranes of Gram negative bacteria. **12c** has a much lower K_D value than **7** (Refer to Table 4). The lower value of r^2 for this batch of results (Table 5) compared with results in Table 4 was due to the greater diversity of structures and K_D values (e.g. $-CF_3$, $-OCF_3$, OH, OCH_3, dinitro compound) and side chain.

Compound **12c** gave better results (64 MIC) against *E. coli* than **7** (MIC 128). The partition coefficient (K_D) of **12c** is lower than that of **7** and further studies of K_D values against MIC values were carried out to investigate the value of this test. Fluorine substitution shows effective enhancement of activity, with excellent results not just on *E. coli*, but also against all bacteria except *E. faecalis*. Compounds **8a** (3-hydroxy), **8b** (4-hydroxy), **8c** (3,4-dihydroxy), **9a** (4-methoxy) and **12c** (refer to Table 5) all gave the same good results against *E. coli*. However, as noted in Table 4, compound **9a** has a larger K_D compared to **7**. This suggested that lipophilicity of the compounds would not be the only factor or the dominant factor in activity against the chosen bacteria and may not apply to all the Gram positive bacteria.

Compound **9a** (4-methoxy substitution) gave excellent results against Gram positive bacteria as did **12c** yet **9a** and has much higher K_D than **12c** (479 against 101). This suggests that low K_D values are not required for high activity against Gram positive bacteria. Interestingly, this is opposite to what was generally observed for the Gram negative bacterium *E. coli*. Compounds **8b** (4-hydroxy) and **49c** (4-trifluoromethyl) have identical results to **9a** with all the microorganisms tested excepted *E. coli* (**49c** has MIC values of 96). Compound **9a** gave much better results (MIC 64) against *E. coli* than compound **50a** (4-trifluoromethoxy) (MIC 512). Fluorine substitution of this type has detracted from activity with other bacteria and the fungus. Comparisons of activity with

Gram positive bacteria demonstrated that the K_D is not the main factor involved in activity against the bacteria as **50b** has a lower K_D than **9a**. The structures are quite different and will govern K_D values.

No improvement in antibacterial efficacy was found by the use of other fluorine containing substituents such as $–CF_3$ and $–OCF_3$. In fact, the latter group was greatly detrimental when substitution at the 4 position to the side chain was effected (compare **49c** with **50b**). At positions C-2 and C-3 the $–CF_3$ detracted from activity against *E. coli*, indicating that this type of substitution may be interfering with the antimicrobial effect, e.g. by blocking access of the reacting species.

Compound **49c** (4-trifluoromethyl) has a similar structure that of **50b** (4-trifluoromethoxy), except for the extra oxygen atom attached to the $–CF_3$. They both gave similar antibacterial results, except for *E. coli*. **50b** has a much higher MIC against *E. coli* (MIC >512) than **49c**, which is expected due to the K_D for **50b** being 155 and being 68 for **49c**. Compound **49c** has quite good activity (MIC 96) and similar to the compounds with fluorine on the aromatic ring.

2.7.3 Other non β-methyl-β-nitrostyrene based compounds

The introduction of a second nitro group by the use of terephthaldehyde [**51**] was also seen to be an important factor for study. Comparisons were made against the standard, unsubstituted aromatic compound [**11**]. The main series of microbiological tests included compounds with the naphthalene [**53a**, **53b**] instead of the benzene ring of β-methyl-β-nitrostyrene. A nitrochromene compound [**54a**] was also tested, along with the fluorine derivative [**54b**]. All the compounds tested, except compounds **10a** and **10b**, showed good to excellent activity against the fungus *Candida albicans*. With regard to the Gram positive bacteria, all the compounds tested showed good to excellent activity with several

[**9a**, **8b**, **49c**, **50b**, **51a**, **52** and **11**] being comparable to **12c**, in accordance with the results of previous studies (Nicoletti *et al*.[29]). Hence, so far, it can be concluded that all of these nitrocompounds appear to belong to a class of compounds which are quite effective as antimicrobial agents.

The two nitropropenyl groups of compound **51**, compared to compound **11**, proved to be detrimental in activity against *E. coli*. A considerably higher K_D was also observed with **51**; however, results for the Gram positive bacteria and the *C. albicans* all were excellent.

All these compounds have large K_D values. Nitrochromene [**54a**] was not active against *E. coli* to any appreciable extent and there was no improvement with fluorine substitution [**54b**]. Generally for *E. coli* inhibition the more hydrophilic compounds, with lower K_D values, performed better than those with higher K_D values.

In summary, fluorine substitution on the ring at position 4 [**12c**] was slightly better than substitution with –OH [**8b**] and $–OCH_3$ [**10b**], $–CF_3$ [**49c**] and no ring substitution [**11**] against *E. coli*. The initial series indicated 1-fluoro-4-(2-nitroprop-1-enyl)benzene as the most active, and all compounds had very good activity (MIC 2-27) against *S. aureus*, *B. subtilis* and *E. faecalis*.

2.8 Results with *E. faecalis* and *E. coli*

By considering *E. faecalis* (Gram positive) and *E. coli* (Gram negative) the influence of substitutents is apparent as the MIC values are higher than for other bacteria and the fungus.

E. faecalis

- $-OCH_3$, -OH, $-CF_3$ at 4-position, $-OCF_3$ at 4-position, the dinitropropenyl compound prepared from terephthaldehyde [**51**], **7** and **12c**, and unsubstituted β-methyl-β-

nitrostyrene [11] all had high activity. There was no obvious benefit of any substitution.

- The best performance (MIC 4) was seen with the 2-naphthyl derivative [53b]. The 1-naphthyl derivative [53a] was distinctly less active but a good result (MIC 32) was obtained against this Gram positive bacterium.
- 3-fluoro and 4-fluoro substitution on the ring [12b, 12c] gave very good results respectively (MIC 16), akin to the results with 7 results and no substitution [11].
- Good results (MIC 32) were also obtained with compound 52 (CH_3 at position 4), the 1-naphthyl derivative [53a].
- The nitrochromenes (MIC both 128) were only moderately active.
- The β-nitrostyrene [1a] compound made from nitromethane was inferior (MIC 128) to the β-methyl- β-nitrostyrene derivative [11] (MIC 16). Activity was improved by substitution at position 4 with fluorine [1b], but not to the extent as with 12c. A methyl group at position 4 [52] showed no antibacterial enhancement (MIC 32 against 16 for the unsubstituted compound, 11).

E. coli

- Compound 7 was not significantly active against *E. coli* (MIC 128).
- Fluorine substitution at position -4 [12c] enhanced activity but fairly good results were obtained without any substitution [11] (MIC 92).
- An –OH group at position -3 or -4, two –OH groups at position 3 and 4, an -OCH_3 at position 4, and –CF_3 at position 4 gave compounds that were all fairly active (MIC 64).
- An –OCF_3 at position 4 [50b] caused a sharp drop in activity compared to the same substituent at position 3 [50a].
- The terephthaldehyde product [51] had extremely poor activity (MIC > 512) in complete contrast with the result against *E. faecalis* (MIC 2).

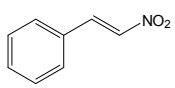
- The product with 2-OCH_3 groups or one with an –OH and -OCH_3 were only of moderate activity [**10a** and **9c**]
- Best results (MIC 64) were obtained with 3-OH [**8a**], 4-OH [**8b**], 3,4-dihydroxy [**8c**], 4-OCH_3 [**9a**], 3,4-dimethoxy [**9b**] 4-fluoro [**12c**], 4-CF_3 [**49c**] and parent compound [**11**].
- Substitution with fluorine at the 3-position [**12b**] reduced the activity of the unsubstituted compound [**11**] (MIC 256 compared with 96).
- Substitution with –CF_3 at position 2 [**49a**] gave a very unsatisfactory result (MIC 512).
- In contrast to the *E .faecalis* results, the naphthyl derivatives were both very poor and the 2-naphthyl derivative [**53b**] (best with *E. faecalis*) gave the worst result of all (MIC>512).
- The nitrochromene derivatives [**54a** and **54b**] were both poor (MIC 256) and both were worse than the unsubstituted compound [**11**] made from nitroethane. Substitution with fluorine was of no consequence.

Against *E. coli*, the most active compounds were compounds **8a**, **8b**, **8c**, **9a** and **12c**; all with MIC values of 64 µg/mL. Results with compound **11** (no substitution) indicated that the basic structure of β-methyl-β-nitrostyrene already has a high activity against this bacterium. Substitution on the ring with –OCH_3, -OH, -CF_3 and –F have only marginally improved this activity.

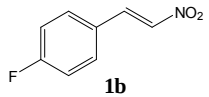
Results against the Gram positive bacteria and the fungus *Candida albicans* were generally good to excellent. More detailed analysis and comparisons follow.

Table 5: Microbiological evaluation of nitropropenyl arenes. Figures are MIC values in µg/mL

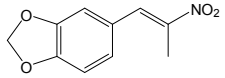
Strain	Compounds and MIC values (µg/mL)												
	1a	**1b**	**7**	**8a**	**8b**	**8c**	**9a**	**9b**	**10a**	**10b**	**11**	**12b**	**12c**
Staphylococcus aureus	256	128	8	13	2	2	2	2	16	16	8	8	8
Bacillus subtilis	256	256	8	6	2	6	2	2	16	16	16	16	8
Enterococcus faecalis	128	64	8	ND	4	ND	4	5	ND	ND	16	16	16
Escherichia coli	256	256	128	64	64	64	64	128	161	81	96	256	64
Candida albicans	32	32	8	19	4	4	4	4	128	128	6	8	4
Partition Coefficients (K_D)	51	62	362	145	150	111	479	250	41	186	113	14	101



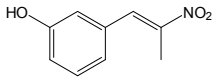
1a



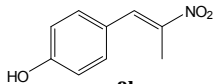
1b



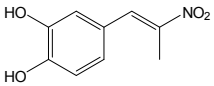
7



8a



8b



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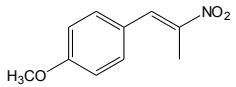
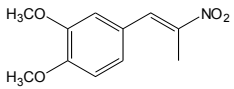
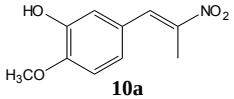


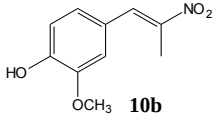
9a



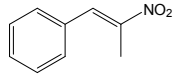
9b



10a



10b



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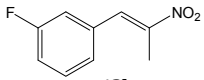
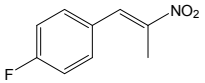


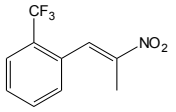
12b



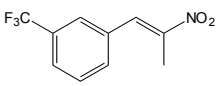
12c

Continuation of Table 5

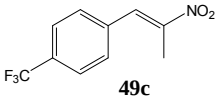
Strain	Compounds and MIC values (µg/mL)											
	49a	**49b**	**49c**	**50a**	**50b**	**51**	**52**	**53a**	**53b**	**54a**	**54b**	**55**
Staphylococcus aureus	16	16	2	16	2	4	8	16	4	64	32	64
Bacillus subtilis	8	8	2	8	4	2	16	16	4	128	64	8
Enterococcus faecalis	32	16	4	16	8	2	32	32	4	128	128	16
Escherichia coli	512	256	96	256	512	>512	128	512	>512	256	256	256
Candida albicans	16	8	4	8	8	2	16	16	4	64	32	8
Partition Coefficients (K_D)	60	30	68	18	155	556	453	1492	2561	280	429	70



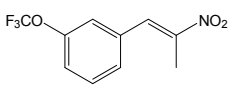
49a



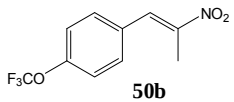
49b



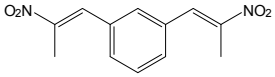
49c



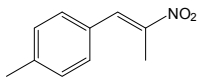
50a



50b



51



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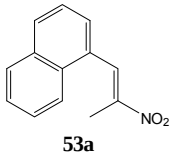
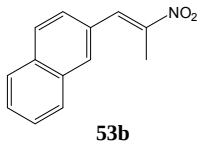
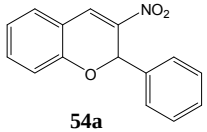


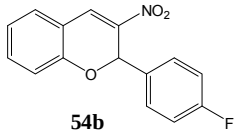
53a



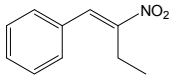
53b



54a

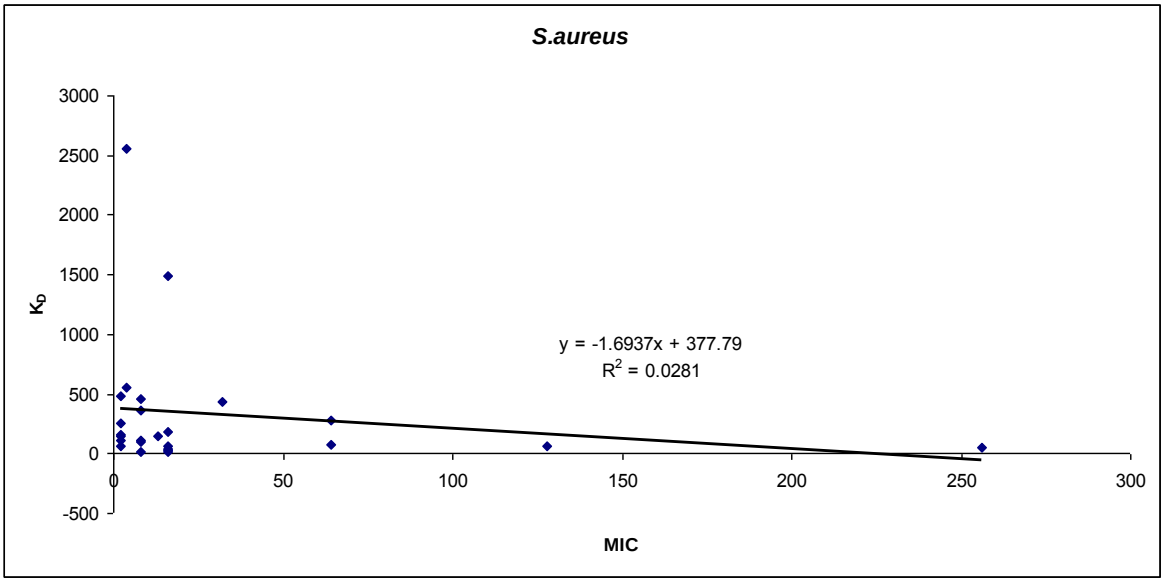


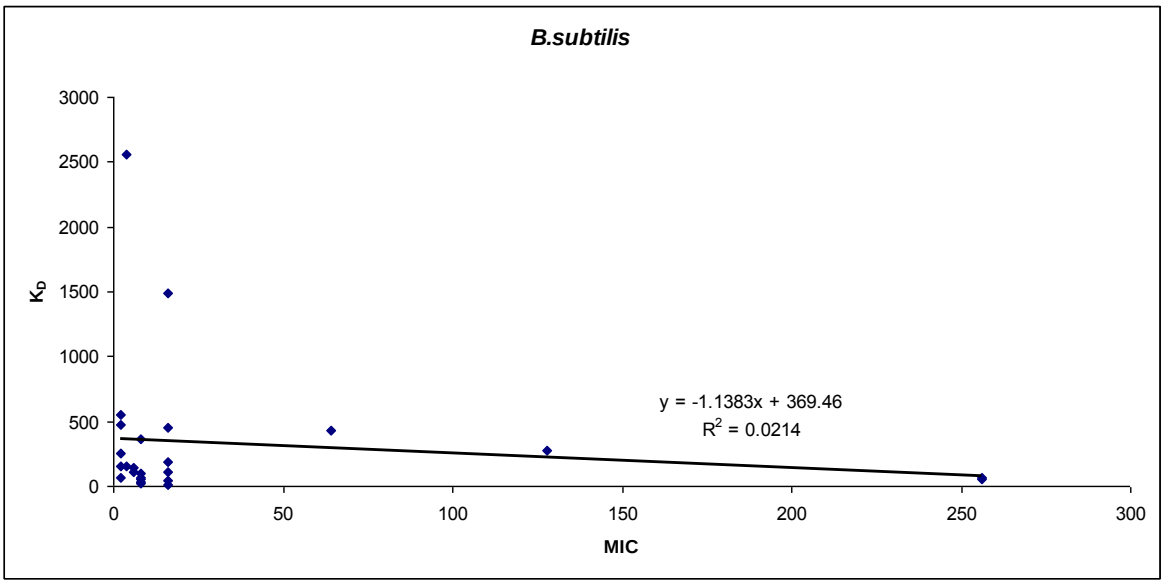
54b

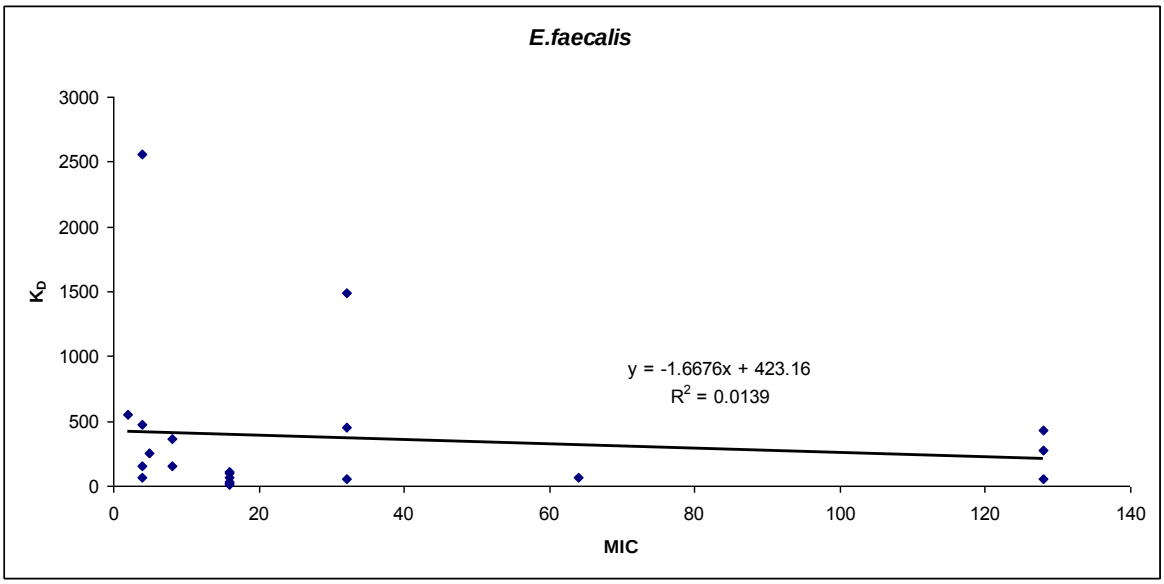


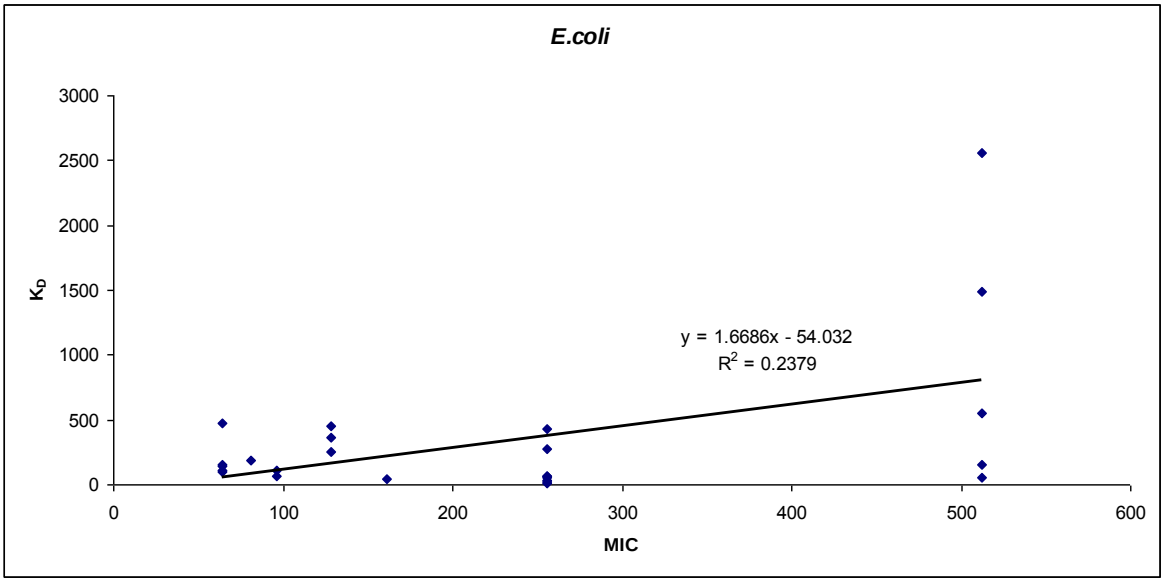
55

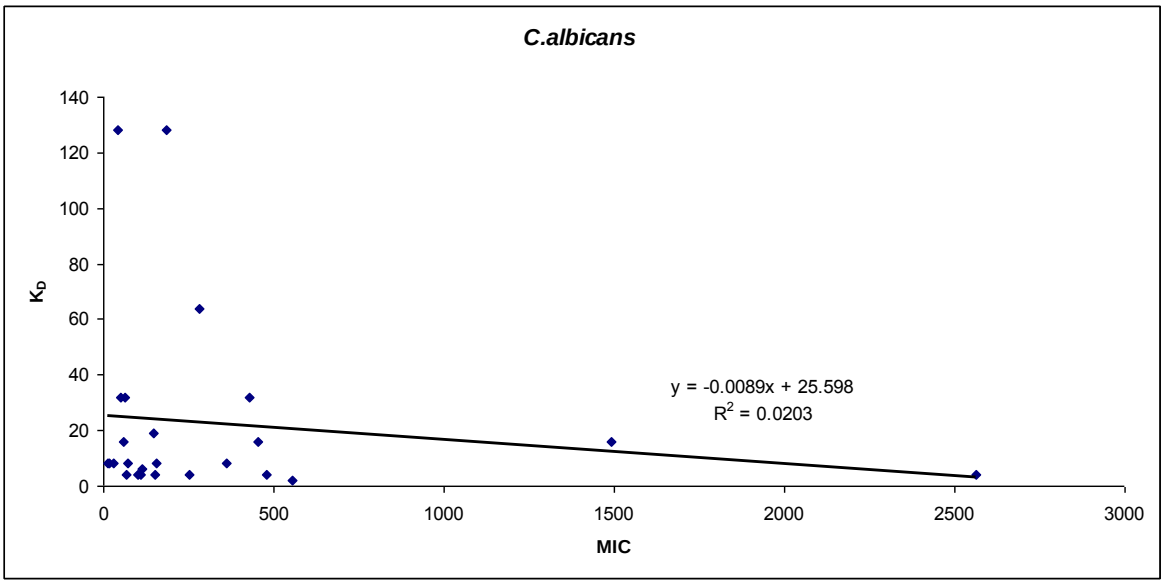
Figure 4: The correlations between MIC and K_D values for each organism






E.faecalis
3000
2500
2000
1500
1000
500
0
K_D
y = -1.6676x + 423.16
R^2 = 0.0139
0
20
40
60
80
100
120
140
MIC


E.coli
3000
2500
2000
1500
1000
500
0
K_D
y = 1.6686x - 54.032
R^2 = 0.2379
0
100
200
300
400
500
600
MIC



Please note that in *C. albicans* K_D-MIC correlations a reversal of the slope/gradient was observerd.

2.8.1 Results with Gram positive bacteria

Results against the Gram positive bacteria (including the previous data of Nicoletti *et al*.[29]) indicated that the growth of this group of bacteria is more readily suppressed by β-methyl-β-nitrostyrene than in the case of *E. coli*. Structure activity relationships were only able to be followed on *Enterococcus faecalis* as nearly all results on *S. aureus* and *B .subtilis* were excellent. In the case of some compounds notably **7**, the compound with $–OCF_3$ substitution at position 4 [**50b**], the terephthaldehyde product [**51**] and a naphthalene derivative [**53b**] there was a complete turnaround from extremely poor results with *E. coli* to extremely good results with *E. faecalis* (MIC values 8 or less). In contrast to the *E. coli* results, the compounds with high K_D values performed much better than those with low K_D values suggesting that the more hydrophobic the compound was the better it performed against *E. faecalis*. Performance of compounds of this type was excellent against the other Gram positive species, *S. aureus* and *B. subtilis* with MIC values all in the range 2-16. Correlation between effectiveness against Gram positive bacteria were only able to be tested against *E. faecalis*. Figure 4 shows that a good correlation was obtained for the

particular compounds tested with negative gradient, the opposite to those with *E. coli*.

2.8.2 Results with *Candida albicans*

For *Candida albicans*, all compounds, irrespective of their activity against the Gram positive or Gram negative microorganisms, performed extremely well with MIC values in the range 2-16, the only exceptions being the following:

- Two hydroxy – methoxy substituted compounds from previous studies [**10a**] and [**10b**] (both MIC 128)

The reason for the lowering of activity of these compounds against *C. albicans*, seen strikingly in the two hydroxy – methoxy compounds [**10a** and **10b**] compared with the two unsubstituted compounds (β-nitrostyrene [**1a**] and β-methyl-β-nitrostyrene [**11**]) is unknown. However, the results of these compounds [**10a** and **10b**] with *E. coli* are also unimpressive and may be due to the effects of two polar groups causing significant blocking of an otherwise interactive site. The 3,4-dihydroxy derivative [**8c**] gave an excellent result and highlights the importance of the positions of substitution in interactions between the compounds and receptor sites on the microorganism.

Substitution of β-methyl-β-nitrostyrene with fluorine at the 4-position [**12c**] resulted in a slight increase in activity against *Candida albicans* (**12c** against **11**). The results of substitution with $–CF_3$ at the 4-position produced no change in activity, but $–OCF_3$ at this position may have attenuated the activity (compare **49c**, **50b** and **11**). The unsatisfactory results with both nitrochromenes [**54a** and **54b**] may be due to blocking by groups in the vicinity of the nitro group (oxygen atom and aromatic ring). These compounds likewise did not perform well against the bacteria.

2.9 Summary of SARs results

2.9.1 Substitutions on aromatic ring

1. –OH vs. $–OCH_3$ (**8b** vs. **9a**)

   The activities were identical with excellent results against all microorganisms except *E. coli*, which gave an MIC of 64 in each case. The result for the methoxy substituted compound is surprisingly good, especially considering its high K_D value (479). The results are somewhat better than the parent compound [**11**].

2. $–OCH_3$ vs. $–OCF_3$ (**9a** vs. **50b**)

   The methoxy substitution proved vastly superior to the trifluoromethoxy group in tests against *E. coli* (MIC 64 against 512). However, in the tests against other bacteria and *C. albicans*, both substitutions were observed to be slightly better than no substitution [**11**], the major difference being that activity against *E. coli* was adversely affected as mentioned above. For this group of compounds, the K_D values were not reliable indicators of activity against *E. coli*.

3. $–CF_3$ vs. $–OCF_3$ (**49c** vs. **50b**)

   The results with $–CF_3$ were excellent against all the microorganisms tested except *E. coli*. The compound [**49c**] still had fairly high activity (MIC 96) making it comparable to one without substitution, but the $–OCF_3$ substitution destroyed the activity against *E. coli*. The K_D value of compound **49c** was only 68 and indicative of its superiority to **50b** (K_D 155) against *E. coli*.

4. $–CH_3$ vs. $–CF_3$ (**52** vs. **49c**)

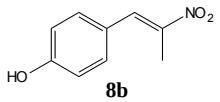
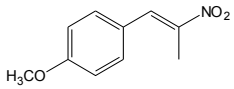
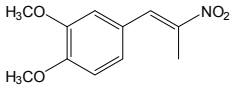
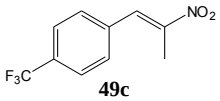
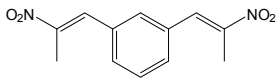
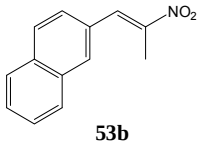
   The methyl group at position 4 gave similar results to the $–CF_3$, suggesting that the fluorine substitution for hydrogen had no significant effect on activity across the range of microorganisms tested despite the huge differences in K_D values.

5. –F vs. $–CF_3$ (**12c** against **49c**)

   The excellent results reported for –F substitution at position 4 was only marginally better than those for $–CF_3$ at the same position. Nevertheless, -F substitution remains the best type for overall high activity against the Gram negative, Gram positive and fungal microorganisms tested.

The Gram positive bacteria tested were most effectively inhibited by the following substitutions shown in Table 6.

Table 6: Most effective compounds against the Gram positive bacteria

Structure	Bacteria (MIC)
 **8b**	***S. aureus* (2)** ***B. subtilis* (2)** ***E. farcalis* (4)**
 **9a**	***S. aureus* (2)** ***B. subtilis* (2)** ***E. farcalis* (4)**
 **9b**	***S. aureus* (2)** ***B. subtilis* (2)** ***E. farcalis* (5)**
 **49c**	***S. aureus* (2)** ***B. subtilis* (2)** ***E. farcalis* (4)**
 **51**	***S. aureus* (4)** ***B. subtilis* (2)** ***E. farcalis* (4)**
 **53b**	***S. aureus* (4)** ***B. subtilis* (4)** ***E. farcalis* (4)**

These products were also the most effective against the fungus *Candida albicans* and were likewise superior to the unsubstituted parent compound.

Relative activities of compounds

The relative activities of some compounds are summarized Table 7.

Table 7: Relative activities of some compounds

Relative activities	Structure
Has the best activity against *E. coli*, and was also excellent against *C. albicans* and very good against *S. aureus* and *B. subtilis*.	**12c**
It does not show results for *E. coli* as good as **12c**, but it is comparable on Gram positive bacteria and fungus.	**7**
Compounds showed excellent activity against the Gram positive microorganisms and the fungus	**8b**, **8c**, **9a**, **9b**, **49c, 50b, 51** and **53b**[#]
These compounds have relatively high activity against *E. coli*	**8a**, **8b**, **8c**, **9a, 9b, 10b, 11, 12c** and **49c**
Very poor activity against *E. coli* (MIC > 512) in contrast to their high activity against the Gram positive bacteria and the fungus	**49a**, **50b**, **51** and **53b**
There was very good agreement on the compounds tested by Nicoletti *et al*.	**7**, **12c**, **8a**, **8c**, **10a** and **10b**

#Note: The K_D values of 556 [**51**] and 2561 [**53b**] were quite the opposite of the low K_D values associated with activity against *E. coli*.

2.10 The effect of different substitutions on lipophilicity

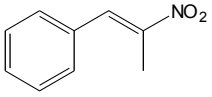
In Chapter 1 lipophilicity was discussed as an indicator of the permeability of a drug to pass through living cellular membranes. In this section, the results of the effect of different substituents on lipophilicity are presented. Substitutions of –F, $-CF_3$, $-OCF_3$, -OH, $-OCH_3$ and the combination of the latter two were studied and are shown in 2.9.1. Figure 5. *E. coli* gave better correlations than the other tested bacteria and fungus even though they were not as good as the correlations shown in Fig 2. It still shows that low K_D values generally work better against *E. coli* than the compounds with higher K_D values.

Although compound activity again did not correlate with K_D values, the Gram positive bacteria did not display good correlations. Compounds with high K_D values are more compatible/effective against the Gram positive bacteria. This is also the case with *C. albicans*.

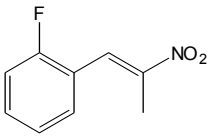
2.10.1 Summary of tested substitutions on β-methyl-β-nitrostyrene

Figure 5: Various substitutions on β-methyl-β-nitrostyrene

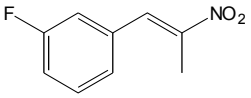
1. Fluorine atom(s) substitution, -F



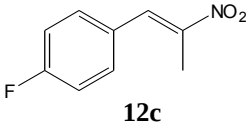
11



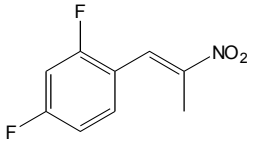
12a



12b

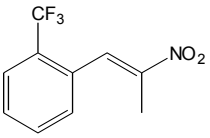


12c

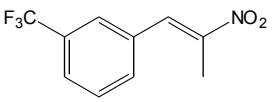


12d

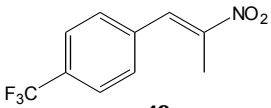
2. Trifluoromethyl substitution, -CF_3



49a

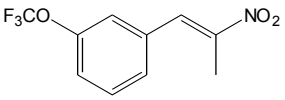


49b

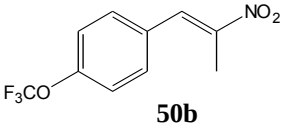


49c

3. Trifluoromethoxy substitution, -OCF_3

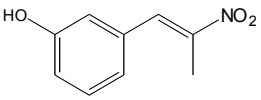


50a

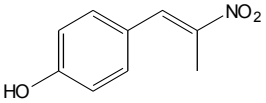


50b

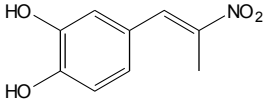
4. Hydroxy substitution, -OH



8a

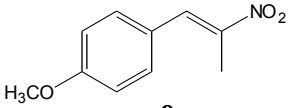


8b

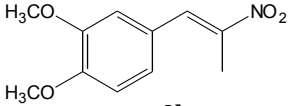


8c

5. Methoxy substitution, -OCH_3

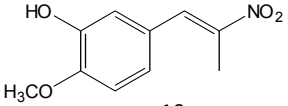


9a

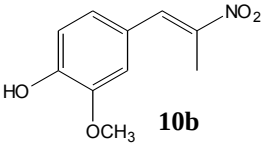


9b

6. Hydroxy and methoxy substitution, -OH and –OCH_3



10a



10b

2.10.2 Summary of results of lipophilicity studies

The results obtained showed, surprisingly, that changing the position (-2, -3, -4) of the fluorine atom on the aromatic ring (**12a**, **12b**, and **12c**, Table 8) altered the lipophilicity of the parent compound [**11**] by either increasing K_D slightly or decreasing K_D. This was especially noted with **12b**, in which the 3-position of substitution gave a very low K_D (14). Compound **12d** with two fluorine atoms substituted on the ring also decreased the lipophilicity of compound **11** by half (K_D 65). However, only **12a** gave a larger K_D value (246, refer to Table 4) than compound **11**. (K_D 113)

The lipophilicity results of compounds **49a**, **49b** and **49c** showed that substitution of a trifluoromethyl group on the ring decreased the K_D value (repective K_D values 60, 30, 68)of the parent compound [**11**] by about a half. Substitution on position 3 gave the lowest K_D value out of the group.

Substitution on position 3 of the ring of a methoxy group [**50a**] again showed an extremely low K_D value (K_D 18), this being lower than other derivatives. Compound **50b** was similar to **12c** both compounds having higher K_D values (K_D 155 and 101) than the parent compound [**11**].

It can be seen by reference to Table 9 that hydroxy group substitutions gave similar results to the fluorine atom but substitution on position 3 [**8a**] still results in a lower K_D value than substitution on position 4 [**8b**] even though the difference of **8a** and **8b** in K_D is not large. The dihydroxy compound remains the lowest K_D (111) of the other two derivatives and has a slightly lower K_D than compound **11**, while **8a** and **8b** did not have lower K_D values than compound **11**.

Substitution on the 4-position showed the same result as previously; a large K_D was obtained that was significantly larger than the parent compound [**11**]. The dimethoxy

substitution [**9b**] gave a product having K_D about half that of the monosubstituted compound [**9a**], and the result is similar to the other two di-substituted compounds (**8c** and **12d**). However, the K_D of **9b** is still greater than compound **11**.

A small K_D (41) was obtained on compound **10a**, in which a hydroxyl group is located at the 4 position of the ring. The value of K_D increased to back over 150 when the –OH group was moved to position 4, which also happened with compound **8b**.

In summary, low K_D values compounds usually mean high efficiency against *E. coli* (Gram negative bacterium), while high K_D values compounds generally are more effective against Gram positive bacteria. For the fungus, all compounds generally have good antibacterial activity even with high or low K_D values. However, the K_D values can only be used as a guide to estimate whether a compound is likely to be effective against the bacteria being tested. Some compounds, for example **12b** and **12d**, have lower K_D values than **12c**, but they do not have comparable activity to **12c** against *E. coli*.

Table 8: K_D values of fluorine-substituted derivatives of β-methyl-β-nitrostyrene

Compound	Substitution	K_D
12a	2-fluoro	138
12b (8a and 10a)	3-fluoro (3-hydroxy and 3-hydroxy-4-methoxy)	14 (145 and 41)
12c (8b)	4-fluoro (4-hydroxy)	101 (150)
12d	2,4-difluoro	65
49a	2-trifluoromethyl	60
49b	3-trifluoromethyl	30
49c	4-trifluoromethyl	68
53	4-methyl	60
50a (10b)	3-trifluoromethoxy (3-methoxy-4-hydroxy)	18 (186)
50b (10a)	4-trifluoromethoxy (4-methoxy)	155 (479)
1a	No ring substitution, no methyl substitution	51
11	Parent compound	113

Table 8 shows K_D values of the various fluoro derivatives tested. Note the low values of K_D as the result of substitution at the 3-position by –F, -CF_3, -OCF_3. Correlation between activity and K_D values were not suggestive of a relationship. Results in brackets are intended for comparison.

Table 9: K_D values of hydroxy and methoxy derivatives of β-methyl-β-nitrostyrene

Compound	Substitution	K_D
7	3,4-methylenedioxy	362
8a	3-hydroxy	145
8b	4-hydroxy	150
8c	3,4-dihydroxy	111
9a	3-hydroxy-4-methoxy	41
9b	3-methoxy-4-hydroxy	186
10a	4-methoxy	479
10b	3,4-dimethoxy	250
11	None	113

Table 9 lists the K_D values of compounds with hydroxy and methoxy substitutions showing the general tendency for K_D to be raised by methoxy, dimethoxy and methylene dioxy substitution. A notable exception is in the case of the 3-hydroxy-4-methoxy derivative. For these compounds, correlations between activity and K_D values were not as strong as previously.

Table 10 provides a summary of the effects of –F, -CF_3 and –OCF_3 substitutions of β-methyl-β-nitrostyrene. The 4-fluoro derivative had the highest activity against *E. coli*, while the 2-fluoro derivative was also of good activity. The activity against the Gram positive bacteria and the fungus were all high except for **49a**, where a –CF_3 group was substituted at the 2-position. Further details can be found in section 2.8

Table 10: MIC values (μg/mL) of β-methyl-β-nitrostyrenes with fluorine-containing substitutions

Compound	Substitution	K_D	*E. coli*	*S. aureus*	*B. subtilis*	*E. faecalis*	*C. albicans*
12a	2-fluoro	138	42	3	3	5	3
12b	3-fluoro	14	256	8	16	16	8
12c	4-fluoro	101	27	2	2	2	2
12d	2,4-difluoro	65	45	4	2	6	3
49a	2-CF_3	60	512	16	32	64	32
49b	3-CF_3	30	256	16	8	16	8
49c	4-CF_3	68	96	2	2	4	4
50a	3-OCF_3	18	256	16	8	16	8
50b	4-OCF_3	155	512	2	4	8	4

2.10.3 The optimal K_D values for activity of β-methyl-β-nitrostyrene derivatives

In this project lipophilicity was used as a guide to identify the optimal K_D values of tested compounds against the chosen bacteria. The results (Table 11), showed that the lower K_D values for fluorinated compounds normally are effective against Gram positive bacteria and fungus. However, only **12c** gave the best result against the Gram negative bacterium *E. coli*. Conversely, the Log *P* range for non-fluorinated compounds was very broad against the Gram positive and fungus. For *E. coli* the range has been narrowed

significantly, possibly due to the structure of each compound being similar to the β-methyl-β-nitrostyrene derivatives. With this we discovered that the lipophilicity of β-methyl-β-nitrostyrene derivatives is not the dominant factor affecting the potency against the chosen bacteria. The structure activity relationships (SARs) would still indicate the major factors that affects the potency.

Table 11: The range of optimal K_D values for activity of β-methyl-β-nitrostyrene derivatives

Microorganism	**Non-fluorinated compounds**		**Fluorinated compounds**	
	Effective K_D range (MIC ≤ 16)	Log *P* range	Effective K_D range (MIC ≤ 16)	Log *P* range
E. faecalis	70-2561	1.85-3.41	14-155	1.15-2.19
S. aureus	41-2561	1.61-3.41	14-155	1.15-2.19
B. subtilis	111-2561	2.05-3.41	18-155	1.26-2.19
E. coli	111-479	2.05-2.68	101	2.00
C. albicans	14-2561	1.15-3.41	45-138	1.81-2.14

2.11 Trends with Gram positive bacteria

The correlations between MIC and K_D values were not straightforward with the Gram positive bacteria, but generally large K_D values were associated with higher activity against these microorganisms. This was in contrast to K_D values of compounds tested against *E. coli*, where lower K_D values were required for high activity. The trends of results in detail are as follows.

2.11.1 Trends with *E. faecalis*

There was no correlation between MIC values and K_D values for *E. faecalis*, even when the compounds were grouped as fluorine-containing and non-fluorine containing types. The r^2 values were 0.0624 and 0.0261 respectively. However, it was apparent that for

each group (excluding those compounds that were not based on β-nitrostyrene alone) the K_D values were higher (mean = 530) for the most active (MIC 2 – 6) compounds. For those compounds with less activity (MIC ≧ 8) the mean K_D was 229.

The compounds with high activity, despite low K_D values (< 70) was **49c** (-CF_3 at position 4), **12d** (2 fluorine atoms at position 2 and 4), **12a** (fluorine at 2 position) were partly due to the influence of fluorine substitution on activity and K_D. All other compounds with high activity had K_D values of ≧ 150. However, the poorest activity (MIC 64) was observed with compound **1a**, without the β-methyl group and the same result was obtained with **1b**, with no β-methyl group. Despite fluorine substitutions, compound **12c** with K_D 101 and MIC 16, was not among the best performers and had about the same activity as the unsubstituted β-methyl-β-nitrostyrene with K_D 113. Generally, substitution with fluorine reduced the K_D values but the extent of this reduction depended upon the position of substitution. For example in compounds **12a**, **12b** and **12c**, substitution at position 3 gave the lowest K_D of 14. However, the highest activity was seen in compound **12a** (position 2 substitution) with MIC 5 and K_D 139 (the highest K_D of the three).

The difference between –CF_3 and –CH_3 was seen with compounds **49c** and **52** both with substitution at the 4 position. The –CF_3 substituted compound was highly active with MIC 4 compared with MIC 32. Substitution of –CF_3 at position 2 [**49a**] resulted in a compound of low activity (MIC 45). With –OCF_3 substitution, compound **50b** (position 4) with K_D 155 and MIC 8 was not quite as active as the methoxy compound with K_D 479 and MIC 4. The best results of all were with compound **51** with two nitro groups having K_D 556 and MIC of only 2.

2.11.2 Trends with *S. aureus*

For –OH and –OCH_3 substitution, the most active compounds against *S. aureus* were the 4-hydroxy [**8b**] substituted compounds of β-methyl-β-nitrostyrene together with the 3,4-dithydroxy [**8c**], 4-methoxy [**9a**] and the 3,4-dimethoxy [**9b**] derivatives. All of these compounds were more active than the unsubstituted parent compound but there was no significant correlation between the MIC and K_D values. Substitution at the 3-position (compound **8a**) was not as effective as substitution of –OH at the 4-position and was of no advantage over the unsubstituted compound. For all non-fluorine substitutions, the only compound of comparable activity to those above was compound **53b**, the 2-naphthyl derivative with K_D 2561 and MIC 4. For the fluorine-containing compounds, the most active were **12d** (2,4-difluoro), **49c** (4-trifluoromethyl) and **50b** (4-trifluoromethoxy), closely followed by **12a** (2-fluoro). However, no correlation between K_D and MIC values was observed. The worst result was with **1b**, the *4*-fluoro-compound without *β*-methyl substitution.

2.11.3 Trends with *B. subtilis*

For –OH and –OCH_3 substitution, the 3,4-dimethoxy derivative, with the highest K_D of 250 was the most active compound. Also of high activity were **8a** (3-hydroxy) and **8c** (3,4-dihydroxy). For all the non-fluorine containing substitutions the results were similar to those of *S. aureus*, with **53b** (2-naphthyl derivative) again showing high activity. For the fluorine-containing derivatives, the results were very similar to those for *S .aureus*, the worst result being with **1b** (4-fluoro derivative but without β-methyl substitution), as it was with *S. aureus*.

In summary, the results for the Gram positive bacteria are quite different from those with the Gram negative bacterium, *E. coli*, and indicate that high K_D values are more likely to be preferable to low K_D values for optimal activity. An example of this is the 2-naphthyl

derivative, (compound **53b**, K_D 2561) which is highly active against all these of the Gram positive bacteria (MIC 4) yet is inactive against *E. coli* (MIC > 512). Compound **12c** is highly active against *E. coli*, but is not among the highest activities against any of the Gram positive bacteria.

Compounds **8b**, **9a**, **9b**, **12a**, **12d** and **53b** are compounds that are highly active against all Gram positive bacteria tested. Compounds **12a**, **12d** and **49c** are the fluorine derivatives that are highly active against these bacteria.

2.12 Trends with Fungus

2.12.1 Trends with *C. albicans*

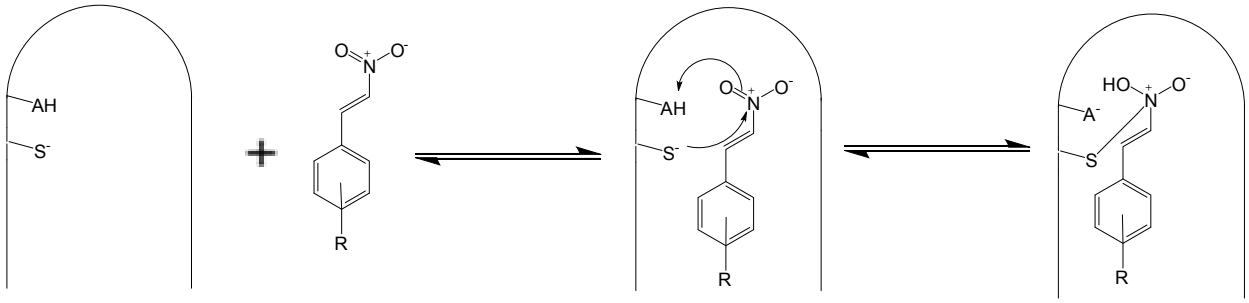
For –OH and $–OCH_3$ substitution, the most active compounds were again the 3,4-dimethoxy and 3,4-dihydroxy derivatives, as was the case for the Gram positive microorganisms. Both showed greater activity than the unsubstituted compound [**11**]. Importantly, the worst compounds were **10a** and **10b** with MICs of 128, yet each of these compounds had one methoxy group and one hydroxy group. The compound without β-methyl group (**1a**) had less activity than compound **11**, but was superior to compounds **10a** and **10b**. For all non-fluorine substitutions, many of the compounds performed well with MIC values of 4. The outstanding compounds (because of poor activity, MIC 128) were **10a** and **10b** as mentioned previously (see last paragraph) **1a** without the β-methyl group (MIC 32) and a nitrochromene (compound **54a**) with MIC 64.There was no significant correlation between MIC and K_D values. For all fluorine-containing compounds, the two of highest activity were compound **12a** (2-fluoro derivative) and **12d** (2,4-difluoro derivative). Fluorination of the nitrochromene made little improvement (**54b** compared with **54a**). Comparison of different positions of the fluorine atom indicated that position 2 may result in better activity than at positions 3 and 4. With regard to the $–CF_3$ group, substitution at position 4 appeared to be the most favorable for activity (MIC 4). The –

OCF_3 group at position 4 gave a product with MIC 2, but this was little different to an –OCH_3 group (MIC 4). The best –fluoro, -trifuoromethyl and –trifluoromethoxy substitutions proved to be more active than the one without substitution [11]. No significant correlation was obtained between MIC and K_D values.

In summary, the results for the fungus, *C. albicans*, are similar to those for the Gram positive bacteria. However, the following points of difference were observed. Compound **8c** (3,4-dihydroxy derivative) was highly active against *C. albicans*, and also highly active against *S. aureus*, but had less activity against *B. subtilis*. Otherwise, all of the compounds which were highly active against the Gram positive bacteria were also highly active against *C. albicans*.

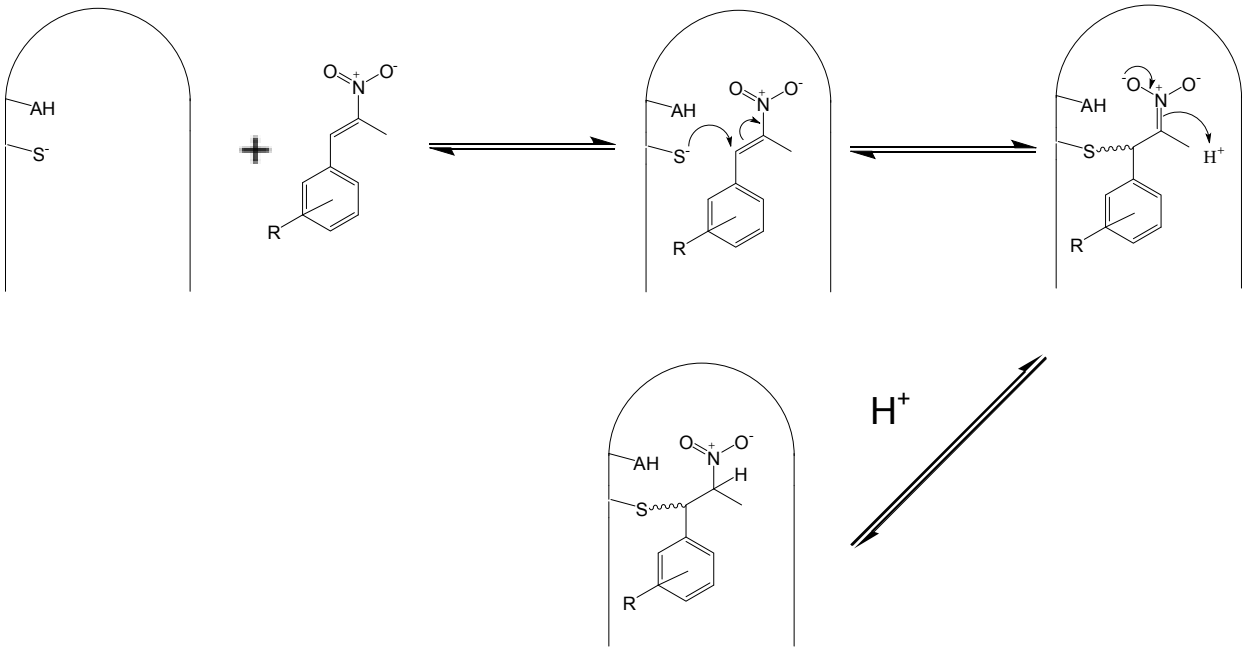
2.13 Mechanism of action

One possible mechansim for activity of these compounds was suggested by Park and Pei[16] who showed that β-nitro-ethenyl benzene (β-nitrostyrene) is a reversible inhibitor of the tyrosine phosphatases PTP1B by means of the formation of a covalent complex with cysteine at the catalytic site. In the absence of free thiol they pictured the selective nucleophilic attack by cysteine on the nitrogroup of β-nitro-ethenyl benzene as the following:



Their mechanistic studies provided the basis for their reasoning. The rationale proposed was that the positive charge on the nitrogen atom, should be particularly reactive to nucleophilic attack, forming a reversible adduct that inhibited PTP1B. However, exactly

the opposite is found in the literature. The conjugate addition to nitroalkenes reflects the high reactivity of nitroalkenes towards nucleophilies[169, 170] and it is widely recognized that nitro group olefins undergo rapid conjugate addition with thiol-type nucleophiles[171-174]. A literature search on the reaction of thiols with nitroarenes found the investigation by Hwu and co-workers[175] who reported that at 185°C for 24hr CH_3SiSNa was able to reduce various aromatic nitro compounds to amines. The susceptibility of the double bond to act as a highly active Michael acceptor for the cysteine nucleophile is for greater than the selective of direct nucleopholic reaction with the nitro group. It is proposed that the following mechanisms could apply[176]. However it does not account for the greater antibacterial potencies of the β-methyl-β-nitrostyrene compounds.



The difference between the cell wall of the Gram positive and Gram negative bacteria is that Gram negative bacteria have an extra layer called the lipopolysaccharide layer[1, 4-6, 9]. The reason why high lipophilicity compounds (e.g. **53b**) can not penetrate into a Gram negative bacterium like *E. coli* is because this high density lipopolysaccharide act as an effective barrier to prevent highly lipophilic agents penetrating the membrane to the interior of the cell[6, 9]. This is a possible rationale why compounds like **53b** work effectively

against the Gram positive bacteria as they do not have this layer and high lipophilicity compounds and are able to penetrate the cell walls of these bacteria.

2.14 Conclusions

The performance of β-nitrostyrene derivatives is governed by the type of substitution on the aromatic ring, as well as by the length of the side chain. According to the tests performed the following conclusions could be drawn:

1. β-nitrostyrene has antibacterial and antifungal activity, but activity against *E. coli* is unsatisfactory.
2. β-methyl-β-nitrostyrene is superior to β-nitrostyrene, showing greater activity to all the microorganisms except *S. aureus*. The β-methyl group confers optimum activity, but a further increase in the size of the side chain results in lower activity.
3. Compound [**7**], with a methylene dioxy ring bridging positions 3- and 4- on the aromatic ring with commercial potential, **BDM-I** (Biodiem Pty Ltd), showed high activity against the Gram positive bacteria and fungus (*C. albicans*) but was unsatisfactory against *E. coli*.
4. Further experiments to investigate the value of substitutions on the aromatic ring with fluorine and fluorine-containing groups such as $–CF_3$ and $–OCF_3$ indicated that fluorine substitution at the 4-position provided the most active derivative (compound **12c**). A general improvement in activity was noted compared with β-methyl-β-nitrostyrene (parent compound).
5. The substitution on the aromatic ring of β-methyl-β-nitrostyrene by hydroxy and methoxy groups produced many compounds with high activity against the Gram positive bacteria and *C. albicans*. However, many of these did not have high activity against *E. coli*. Compounds with comparable activity to the 4-fluoro compound [**12c**] across the range of microorganisms tested were the 4-methoxy

[**9a**] and 3,4-dimethoxy [**9b**] derivatives. Combinations of hydroxy and methoxy were not quite as effective and the positions of substitution were important factors.

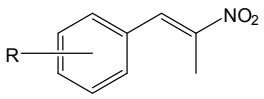
6. Compound **53b**, featuring a naphthalene substitution was interesting in that it gave excellent results against all the Gram positive bacteria and *C. albicans*, but failed badly against *E. coli*. It had a high K_D value (2561), which seems to be desirable for activity against Gram positive bacteria.
7. Compounds with lowest K_D values appear to be more effective against *E. coli* than those with high K_D values and high degrees of correlation were obtained in most cases.
8. Other compounds which performed well against Gram positive bacteria and *C. albicans* was compound **51**, with two nitropropenyl groups. However, it was completely ineffective against *E. coli*.
9. Other fluorine derivatives which also performed well against Gram positive bacteria and *C. albicans* were compound **49c** with substitution of $–CF_3$ at position 4 and compound **50b**, with substitution of $–OCF_3$ at the same position. The latter compound was completely ineffective against *E. coli*.
10. The requirements for high activity against *E. coli* are vastly different from those against the Gram positive bacteria. Differences are also seen with *C. albicans*, but the results against this fungus are much closer to those against the Gram positive bacteria than the *E. coli*.

Compounds that were not simple derivatives of β-methyl-β-nitrostyrene, i.e. compounds, **51**, **54a**, **54b** were of interest, but only compound **51** was in the higher activity bracket. The compound with two nitropropenyl groups is extremely interesting as it had high activity against all microorganisms tested except *E. coli*. It had a K_D of 556, again reinforcing the view that higher K_D values are often associated with higher activity against the Gram positive bacteria. Further work will evaluate compounds with other type of

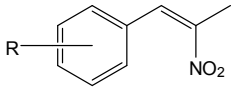
structures such as β-lactams, macrolides, and so on… Therefore it will then be possible to compare the β-nitro arenes with the antibiotics already in use.

2.15 *E*/*Z* configurations of the tested compounds

The *E* and *Z* configurations of a compound is always important in Medicinal Chemistry as either of them can be the key conformation to enhance the drugs efficiency. Most of the compounds in this project are based on β-methyl-β-nitrostyrene except **1a**, **1b**, **55a** and **55b**.

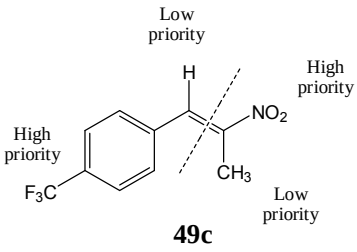


(*E*)-β-methyl-β-nitrostyrene



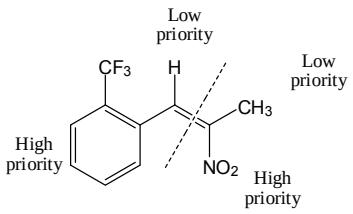
(*Z*)-β-methyl-β-nitrostyrene

The definition of *E* and *Z* configurations depends upon the assignment of priorities to double bond substituents based on Cahn-Ingold-Prelog, priority rules[177]. For example consider an alkene which the two high-priority groups are on the opposite side of the double bond, the compound will be assigned as *E* configuration. The *Z* configuration is when high priority groups are on the same side of the double bond. According to the NMR obtained for each β-methyl-β-nitrostyrene derivatives the *E* configuration was the dominant conformation. The ratio of *E* to *Z* for example in **49c** is 14:1 and the ratio of *Z* configuration would decrease after recrystallization, as the *Z* compound is more soluble in the solvent (95% alcohol). However, an exceptional case was obtained in **49a** where the *Z* configuration is the dominant structure (approx. 1:1.8). As **49a** existed as a liquid, recrystallization can not apply to this compound to minimize the ratio between the *E*/*Z* configurations unless submitting the sample to other analytical instruments (e.g. HPLC) to separate the two isomers. According to the structure of most of the major compounds, the *E* configuration domination is due to two high-priority groups, which are the phenol and nitro group (take **49c** as an example), are on the opposite side of the double bond except in **49a**.



49c

***E* configuration**



49a

***Z* configuration**

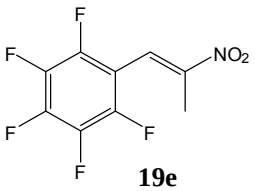
2.16 Substrate for nitrostyrene formation via Henry reaction protocol

Two compounds that have been synthesized, but not yet been tested were **1c** and **56b** (See Chapter 3). They certainly can be tested in the future; however, the aim for making them is they are precursors for future synthesis of other novel nitrostyrene compounds.

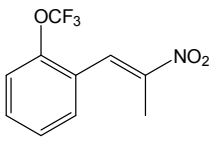
2.17 Future work

There are some compounds that should be investigated for biological testing. Some of them are fluorinated compounds, some of them are the novel compounds and some of them would be designed to compare their biological activity with the existing compounds. (Compounds with * indicates new substances)

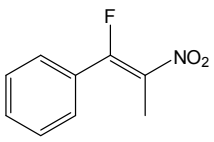
2.17.1 The fluorinated compounds



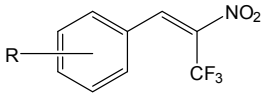
19e



50c*



57*

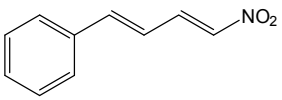


61*

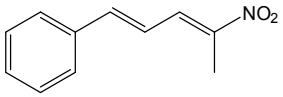
Additionally **19e** should be made for biological evaluation. Compound **50c*** could be used to complete the comparison of antimicrobial activity with substitution series of –F, -CF_3 and -OCF_3.

Compound **58*** and **62*** could be used to test whether replacing the proton by fluorine atom(s) would still affect the biological activity.

2.17.2 Chain extension compounds



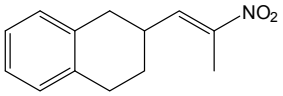
58a



58b

These two chain extended compounds can be used to prove the chain length of the parent compounds are the ideal length for biological activity supporting Milhazes *et al*.[22] theory.

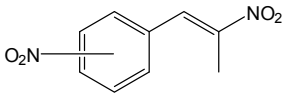
2.17.3 New compounds for comparison purposes



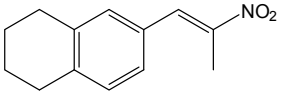
62*

Although a similar compound to the above structure has been made before, such a compound with saturated six- membered ring needs to be tested.

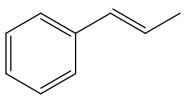
2.17.4 Existing compounds for comparison purposes



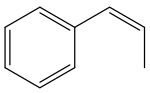
63



64



65a



65b

These known compounds can be found in the literature and again obvious results could be seen from them and we can use them to show that β-methyl-β-nitrostyrene derivatives are one of the most effective types of antibacterial agents.

Chapter 3

3 Experimental

3.1 General Methods and Conditions

3.1.1 Octanol-water Partition Coefficients

The lipophilicity level of each compound was determined by octanol-water partition coefficients. The buffer solution used in the determination of K_D was made by mixing sodium chloride (3.78g, 65mmol), disodium hydrogen orthophosphate (2.14g, 18mmol) and sodium dihydrogen orthophosphate (0.78g, 5.5mmol) in 500mL water at room temperature (~22℃) and had a pH of 7.5. Each compound (10mg) was dissolved in octanol (2mL) in a stoppered test tube, followed with the addition of the buffer solution (2mL) and the tube was shaken over 48 hours and finally the mixtures were allowed to separate into two layers. The top layers were removed and absorbance measured after dilution 1:20, 1:50 or 1:200 to 3mL with octanol in a 1 cm path length apart cuvette at 370nm for each diluted sample. The aqueous bottom layers were removed and the absorbance measured without dilution.

K_D measurements were according to the equation:

$$K_D = \frac{[\text{Octanol}]}{[\text{Water}]}$$

As the absorbance of the octanol and water layers is directly proportional to the concentration in each layer, the K_D value can be calculated from the relative absorbance of each layer.

The calculated log P values (C log P) were obtained using Marvin Sketch (ChemAxon); and a table of all structures with measured and calculated log P values can be referred in Appendix section.

3.1.2 Analysis and instruments

Gas Chromatography and Mass Spectroscopy (GC/MS) spectra were obtained in either electron ionization (EI) or positive/negative electrospray (ESI) modes with the Varian Saturn 2200 GC/MS/MS (ion-trap) coupled to a Varian CP-3800 GC (FactorFour – Capillary Column; Stationary Phase: VF-5ms; L(m) ID(mm) x OD (mm): 30 x 0.25 x 0.39) or Micromass Platform II ESI/MS (240 V, 10 A).

Melting points of the products were determined on a Gallenkamp melting point apparatus and are not corrected.

^{1}H and ^{13}C NMR spectra were determined using a Bruker Advance 300 MHz or Bruker Avance 300 III MHz spectrometer. All spectra were obtained and interpreted using TopSpin v2.0. Some FIDs from Bruker Advance 300 MHz were processed using Mestrec23. All samples, except **8b** which was dissolved in DMSO, were dissolved $CDCl_3$. Proton (^{1}H) chemical shifts were recorded as δ values in parts per million (ppm) downfield shifts; the reference peak is a singlet at 2.78 ppm for DMSO and singlet at 7.26 for $CDCl_3$. Chemicals shifts are presented in multiplicity, coupling constant(s) (*J* in Hz), integration and assignments. Abbreviations are: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, dd = doublet of doublet, m = multiplet. Carbon (^{13}C) chemical shifts were ^{1}H decoupled, and recorded as δ values in parts per million (ppm). Reference peak for DMSO is a massive multiplet at 40.0 ppm and triplet at 77.0 for $CDCl_3$. Additional information to assist assignments of peaks are from CH COSY spectra, gCOSY, DEPT 45, 90, 135, HMBC and HSQC.

3.2 Materials

Organic reagents, solvents and purification reagents were purchased from Ajax Finechem Pty Ltd, BDH, Chem Supply, Merck and Sigma Aldrich and all were of AR quality or better than 99% purity. Results of compounds **7**, **8a**, **8b**, **10a** and **10b** were from Nicoletti *et al*.[29] and for comparison and completeness. Compounds **7**, **8b**, **8c**, **9a**, and **10b** were prepared by HIGH FORCE Research Ltd U.K.

Microbial stocks

The strains used for biological tests were: *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231. The microbial stocks were kept under -80°C in MHB (Oxoid, Cambridge, UK). The antibiotics used as a control for biological testing were erythromycin, tetracycline (Sigma Aldrich, St-Louis, Mo, USA) and ciprofloxacin (MP Biomedicals, Irvine, CA, USA). The nitrostyrene derivatives were diluted and stored in the dark room at room temperature for a month and maintained inhibitory potency when assayed by measurement of the MIC in bacterial species.

3.3 Minimum inhibitory concentrations

The microbiology testing and dilution was based on the National Committee for Clinical Laboratory Standards methods in MHB (Oxoid) for bacteria or Sabouraud Liquid Medium (SLM, Oxiod) for the fungus. *C. albicans*. Microplates assays were performed in clear, round-bottomed, 96-well plates (Sarstedt Australia, SA, AUS) with a total volume of 200 µL per well. The densities of inoculums were estimated by suspension turbidity using McF0.5 standard. Standard inoculums densities for bacteria were approximately 1 x 10^5 Colony Forming Units per mL (CFU/mL) and 1 x 10^4 CFU/mL for *C. albicans*[7].

Inoculum densities were confirmed by serial dilution plating onto NA and incubation aerobically at 37°C for 24 hrs. The tested nitrostyrene derivatives were added to plates at two times tested concentrations in 100 µL media. Ciprofloxacin was used as an internal positive control for bacterium and Miconazole for *C. albicans*. Microplates were incubated 18-24 hrs at 37°C aerobically before reading wells visually for turbidity. All assays included duplicated wells and were at least twice replicated. The geometric MIC (µg/mL) for each strain was adjusted to the nearest $\log_2$ dilution tested. MIC results were reported as MIC (µg/mL) for standards.

3.4 Synthesis of nitroprop-1-enyl-benzene series

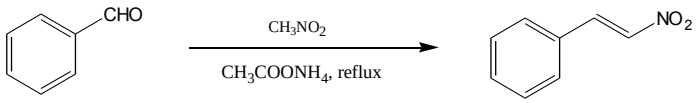
The standard Henry reaction has been used as Method A and B as well as other methods from previous work[29]. Each method had a different reaction time as well as having used different reagents in the reaction, but nitroethane was common to all, except for β-nitrostyrene, where nitromethane was used.

Standard Henry Reaction (Method A)

The first method used was by Knoevenagel[166] where condensation was carried at room temperature in the presence of aliphatic amines such as methylamine. The reaction time required was much longer than for Method B, but no heating was required. Overall yield was often lower than from Method B, therefore most of the compounds were synthesized using Method B. Method B was based on Gairaud and Lappin's[178] method of making nitrostyrene compounds.

3.4.1 Synthesis of β-Nitrostyrene

β-Nitrostyrene/2-nitroeth-1-enylbenzene [1a]



1a

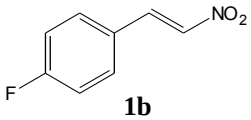
The synthesis procedure was based on Black *et al*.[179]. Benzaldehyde (1.04g, 9.8mmol) was added to a stirring solution containing ammonium acetate (0.20g, 2.6mmol) and nitromethane (5.68g, 93.44mmol). The mixture was heated at reflux (90°C) for 6 hours, poured to water (100mL) and extracted with diethyl ether (3 x 30mL). The organic extract was washed with saturated brine solution (25%, 100mL), dried over magnesium sulphate ($MgSO_4$), filtered and concentrated under high vacuum. The residue was purified by recrystallization from ethanol (95%) to give yellow needles of compound **1a** with a yield of 82% and melting point 58-59°C (Lit. 58-59℃)[179]

^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 7.96-7.91 (d, *J* = 13.7 Hz, 1H, C=αC-H), 7.54-7.49 (d, *J* = 13.7 Hz, 1H, C=βC-H), 7.43-7.38 (m, 5H, Ar-H).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 139.1 (C=C, α carbon), 137.1 (Ar), 132.2 (C=C, β carbon), 130.1 (Ar), 129.4 (Ar), 127.1 (Ar).

GC/MS *m/z* (M^+) 149.

4-Fluoro-β-nitrostyrene [1b]



1b

The synthesis method used was that by Côté *et al*.[180] which was modified from Andrey *et al*.[181]. A stirred solution of acetic acid (33.5mL) and ammonium acetate (4.4g, 57.1mmol) was added to nitromethane (10g, 164.0mmol) followed by 4-fluorobenzaldehyde (2.94g, 23.7mmol) and the solution was refluxed in an oil bath at 100°C for 5hours and 30 minutes. The dark orange mixture was then cooled to room temperature and poured into water (100mL). The pH of the mixture was then regulated to 7 using adding sodium hydroxide solution (2M), after which the product was extracted with ethyl acetate (3 x

100mL). The combined organic extracts were dried over $MgSO_4$ then filtered and concentrated under high vacuum. Further purification was done by recrystallizing the compound from 95% ethanol to remove the brown oily impurities, resulting in obtaining pale yellow needles with yield 57%, and melting point 99-100°C (Lit. 98-100℃)[182].

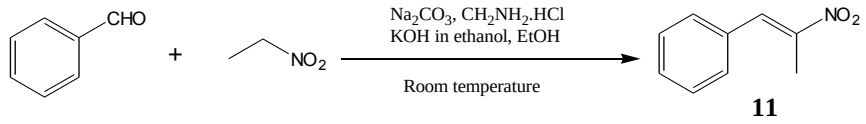
^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 7.94-7.89 (d, *J* = 13.8 Hz, 1H, αC-H), 7.52-7.44 (m, multiplet occurred due to the peaks have overlapped with peaks in the ring, 1H, βC-H), 7.52-7.44 (m, multiplet occurred due to the peaks have overlapped with peaks in the β carbon, 2H, Ar-H), 7.11-7.05 (t, *J* = 8.4, 2H, Ar-H).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 164.9 (d, *J* = 255.1 Hz, C-F), 137.8 (C=C, β carbon), 136.8 (C=C, α carbon), 131.2 (Ar), 126.3 (Ar), 116.7 (Ar).

GC/MS *m/z* (M^+) 167.

3.4.2 Synthesis of β-methyl- β-nitrostyrene

2-Nitroprop-1-enyl benzene [11]



11

The synthesis of this compound was performed by Professor Hugh Cornell who used Method A. To benzaldehyde (2.12g, 20mmol), was added nitroethane (1.8g, 24mmol), anhydrous sodium carbonate (0.3g, 3mmol), methylamine hydrochloride (0.15g, 2.2mmol) with potassium hydroxide (0.05g, 0.9mmol, dissolved in 1.0 mL ethanol) and 2.5mL ethanol. The mixture was reacted at room temperature with sufficient stirring for 44 hours. After that, the mixture was dissolved in hot ethanol (95%) and the hot solution decanted and cooled to 5℃ for 2 hours. The dried crude product was filtered and air dried to give yellow crystals produced with yield 22% and melting point from 60-62℃. The crude product was then recrystallized from 3mL ethanol (95%), washed with 2 x 0.5 portions of chilled ethanol. Purified yellow crystals were obtained in 9% yield with melting point 63-64℃ (Lit. 64℃)[183].

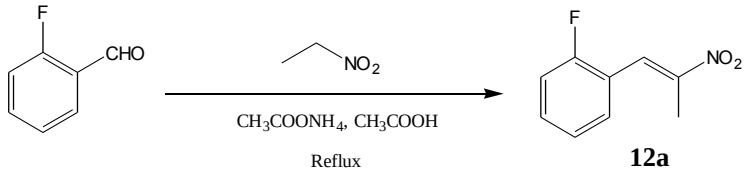
^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 8.10 (s, 1H, H-C=C), 7.46 (s, 5H, Ar-H), 2.47 (s, 3H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 147.7 (C=C, β carbon), 133.5 (C=C, α carbon), 132.4 (s, C=C-C in Ar), 129.9 (Ar), 129.7 (Ar), 128.9 (Ar), 14.0 (CH_3).

GC/MS *m/z* (M^+) 149.

3.4.3 Synthesis of monofluoro substitution product of β-methyl-β-nitrostyrene

1-Fluoro-2-(nitroprop-1-enyl) benzene [12a]



12a

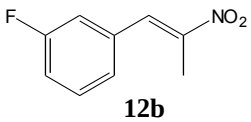
Method B was used for this reaction. 2-Fluoro-benzaldehyde (4.81g, 38.8mmol) was dissolved in nitroethane (4.01g, 53.5mmol, 20% excess), ammonium acetate (4.00g, 52mmol) and glacial acetic acid (5mL) were added and the mixture was refluxed for 2 hours in an oil bath at 100°C. The orange coloured mixture was then chilled and de-ionized water (6mL) was then added. A small portion of the crude orange crystalline product obtained by filtration was taken for determination of melting point. The rest of the product was dissolved in hot ethanol (95%, 2ml) and chilled for an hour to obtain the recrystallized product. The recrystallization process was repeated and light yellow crystals were obtained (Compound **12a; ** 2.38g, 34% of theoretical yield). Melting points were 1st crude: 42- 43°C, 2nd crude: 43- 44°C and final product 45-47°C.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 7.98 (s, 1H, H-C=C), 7.39-7.33 (t, *J* = 8.7 Hz, 1H, Ar-H), 7.19 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.12 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.04 (d, J = 8.8 Hz, 1H, Ar-H), 2.36 (s. 3H,C-H, *E*), 1.59 (s, 3H, C-H, *Z*).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 165.1 (d, *J* = 252.2 Hz, C-F), 147.5 (C=C, β carbon), 132.5 (C=C, α carbon), 132.2 (Ar), 132.0 (Ar), 128.7 (Ar), 116.4 (Ar), 116.2 (Ar), 14.0 (CH_3); **GC/MS** *m/z* (M^+) 181.

Similar procedures were repeated as described above to obtain compounds. **12c**, **12d**, **53a** and **53b**.

1-Fluoro-3-(nitroprop-1-enyl) benzene [12b]



12b

This compound was prepared with a method similar to of Werbal, L. M. *et al*.[184] by reacting 3-fluorobenzaldehyde (1g, 8.05mmol) with ammonium acetate (0.19g, 2.4mmol) in nitroethane (4.98g, 66.4mmol), under reflux overnight (approximately 17 hours) in an oil bath at 125°C. The compound was identified by thin layer chromatography (TLC). The mixture was then concentrated under high vacuum to remove the excess nitroethane and then the yellow mixture was dissolved in chloroform (20ml), washed with water (3 x 20mL) and with sodium chloride solution (25%, 20mL). The mixture was dried ($MgSO_4$) and concentrated under high vacuum. A yellow liquid was obtained (0.75g) this being 52% of theoretical yield.

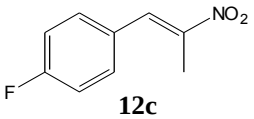
^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 8.04 (s, 1H, C=C-H), 7.46-7.39 (m, multiplet due to proton peaks in the ring coupled with other peaks in the ring, 1H, Ar-H), 7.46-7.39 (m, multiplet due to proton peaks in the ring overlapped with other peaks in the ring and with a F atom, 1H, Ar-H), 7.22-7.19 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.13-7.09 (d, *J* = 9.3 Hz, 1H, Ar-H coupled with F), 2.42 (s, 3H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 164.3 (d, *J* = 246.8 Hz, 1C, C-F), 148.7 (C=C, β carbon), 134.6 (Ar), 132.0 (Ar), 130.6 (C=C, α carbon), 130.5 (Ar), 125.9 (Ar), 125.7 (Ar), 13.9 (CH_3).

GC/MS *m/z* (M^+) 181.

Similar procedures were repeated as described above to obtain compounds: **12e**, **49a**, **49b**, **50a** and **50b**.

1-Fluoro-4-(nitroprop-1-enyl) benzene [12c]



12c

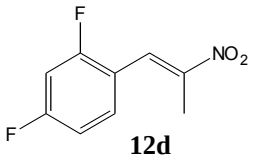
The product was obtained as yellow crystals with 30% of theoretical (2.13g). Melting points: 1st crude: 38°C, 2nd crude: 45°C and final product: 65-66°C[154].

^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 8.06 (s, 1H, H-C=C), 7.46 (dd, *J* = 3.3, 5.4 Hz, 2H, Ar-H), 7.17 (t, *J* = 8.7 Hz, 2H, Ar-H), 2.46 (s, 3H, C-H, *E*), 1.59 (s, 3H, C-H, *Z*).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 165.2 (d, *J* = 252.2 Hz, 1C, C-F), 147.5 (C=C, β carbon), 132.5 (C=C, α carbon), 132.2 (Ar), 128.5 (Ar), 128.5 (Ar), 116.3 (Ar), 116.0 (Ar), 14.0 (CH_3)

GC/MS *m/z* (M^+) 181.

1,3-Difluoro-4-(nitroprop-1-enyl) benzene [12d]



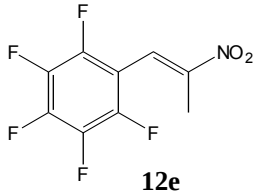
12d

This compound was prepared by the same method as **12d** (Method B) making use of the Henry reaction. Quantities of chemicals used were: 2,4-difluorobenzaldehyde (0.78g, 5.4mmol), nitroethane (0.46g, 5.2mmol, 20% excess), ammonium acetate (0.80g, 10.0mmol and glacial acetic acid (1mL). The mixture was refluxed as before for 2 hours in an oil bath at 100°C. **12d** was obtained as yellow crystals with a melting point of 48-49°C (0.47g, 43% of theoretical yield). **^{1}H NMR** (300 MHz, $CDCl_3$): δ_H (ppm): 8.06 (s, 1H, C=C-H), 7.45 – 7.35 (dd, *J* = 8.3, 8.4Hz, coupling due to two fluorine atoms were coupling with this proton. 1H, Ar-H); 7.04 – 6.96 (dd, for the proton at the middle of two fluorine atoms it should split to a quartet as two fluorine atoms were coupling with it. *J* = 8.5, 8.5 Hz. 1H Ar-H. The other proton at position 6 should be a triplet as there is only fluorine atom coupling with it. t, *J* = 8.5 Hz, 1H, Ar-H), 2.38 (s 3H, CH_3, *E*), 1.57 (s 3H, CH_3, *Z*).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 163 – 162.3 (quartet due to two fluorine atoms coupling with the carbon. *J* = 222.1, 233.6Hz. 1C, F-C=C-C-F), 162.2 – 159 (q, *J* = 234,4Hz. 1C, F-C=C-C-F), 149.5 (s, C=C α carbon), 134.1 – 131.1 (quartet due to two fluorine atoms coupling with the carbon. *J* = 234.6 Hz, 1C, F-C-C=C-C=C-F), 125.7 – 125.4 (s, C=C, β carbon), 117.0 – 116.5 (d, *J* = 9.9Hz, 1C, F-C=C), 112.3 – 111.7 (q, *J* = 3.6, 21.7, 24.9Hz, 1C, F-C-=C-C-F), 104.8 – 104.4 (d, *J* = 25.8, 1C, F-C-C=C), 14.2 – 14.0 (CH_3).

GC/MS *m/z* (M^+) 199.

Pentafluoro-2-(nitroprop-1-enyl) benzene [12e]



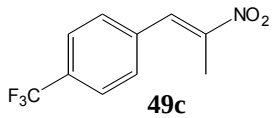
12e

The scale of chemicals used in the reaction was 1/50 to what Werbal, L. M. *et al*.[184] used. A mixture of pentafluorobenzaldehyde (1g, 5.1mmol) and ammonium acetate (0.12g, 1.53mmol) in nitroethane (3.15g, 42mmol) was heated (120°C) under reflux for 17 hours. The large excess of nitroethane was removed under high vacuum. The residue was dissolved in chloroform (10mL) and the mixture was then washed with water (4 x 20mL) and with saturated brine solution (25%, 2 x 20mL). The chloroform solution was dried over $MgSO_4$, filtered and concentrated under high vacuum. The product was purified by flash column chromatography on silica gel with 30% ethyl acetate in hexane (v/v) to obtain a yellow liquid in yield of 2.5%.

GC/MS *m/z* (M^+) 253; (the amount of compound was only enough for GC/MS characterization).

3.4.4 Synthesis of trifluoromethyl substitution of β-nitrostyrene

1-Trifluoromethyl-4-(nitroprop-1-enyl) benzene [49c]



49c

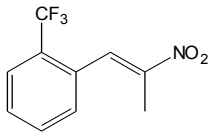
Compound **49c** was synthesized by a method similar to that of Bergner and Opatz[185]. 4-Trifluoromethylbenzaldehyde (1g, 5.7mmol) and ammonium acetate (0.38g, 5.0mmol) were dissolved in nitroethane (20mL, 280mmol), heated to 100°C and refluxed overnight. The excess nitroethane was removed under high vacuum and the yellow coloured mixture was poured into water (20mL) and extracted with ethyl acetate (3 x 20mL). The combined organic extracts were washed with water (3 x 20mL) and sodium chloride solution (25%, 20mL) and then dried over anhydrous $MgSO_4$. The solvent was removed under high vacuum (enhanced with liquid nitrogen) to yield a yellow solid (1.16g, 87% yield). The material was purified by washing with cold ethanol (95%) to yield **49c** (0.748g, 42%), a yellow solid which had a melting point of 96-98°C (Lit. 36-38.5°C)[186].

^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 8.02 (s, 1H, C=C-H), 7.65 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.46 (d, *J* = 8.2 Hz, 2H, Ar-H), 2.37 (s, 3H, C-H, *E*), 1.59 (s, 3H, C-H, *Z*).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 148.9 (s, C=C, β carbon), 135.6 (Ar), 132.6 (s, C=C, α carbon), 131.4 (s, Ar-C-CF_3), 129.8 (Ar), 128.5 (Ar), 123.7 (q. *J* = 273.6 Hz, CF_3), 13.6 (CH_3).

GC/MS *m/z* (M^+) 231.

1-Trifluoromethyl-2-(nitroprop-1-enyl) benzene [49a]



49a

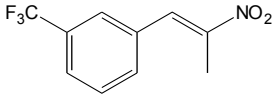
The method of synthesis was similar to compound **12b**. The quantities of chemicals used were: 2-trifluoromethylbenzaldehyde (1.0g, 5.7mmol), ammonium acetate (0.13g, 1.7mmol) and nitroethane (3.52g, 47mmol) were refluxed as before for 19 hours in an oil bath at 125°C. The compound was purified by flash column chromatography on silica gel with hexane/ethyl acetate (20/1) to obtain a yellow liquid in a yield of 46%. For this compound the relative amount of the *cis* and *trans* isomers are 60% and 40% respectively.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 8.16 (s, 1H, C=C-H), 7.69-7.67 (d, *J* = 7.8 Hz, 1H, Ar-H, *trans*-compound), 7.61-7.53 (m, multiplets due to overlap of the *cis* and *trans*-compounds, 1H, Ar-H), 7.48-7.42 (m, multiplets due to overlapped with the *cis* and *trans*-compounds, 1H, Ar-H), 7.39-7.34 (t, *J* = 6.6 Hz, *cis*-compound, 1H, Ar-H), 7.28-7.26 (d, *J* = 7.5 Hz, *cis*-compound, 1H, Ar-H), 7.17-7.15 (d, *J* = 6.3 Hz, *trans*-compound, 1H, Ar-H), 2.31 (s, *trans*-compound, 3H, CH_3), 2.17 (s, *cis*-compound, 3H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 149.9 (s, 1C, C=C, β carbon), 148.9 (Ar), 132.0 (Ar), 130.2 (s, C=C, α carbon), 130.0 (*cis* Ar), 129.3 (Ar), 129.0 (*trans* Ar),128.4 (*cis* Ar),127.2 (q, J = 264.5 Hz,1C, CF_3), 126.4 (*trans* Ar), 125.9 (Ar), 124.7 (Ar), 122.0 (Ar), 19.3 (s, 1C, *trans* CH_3), 13.7 (s, 1C, *cis* CH_3).

GC/MS *m/z* (M^+) 231.

1-Trifluoromethyl-3-(nitroprop-1-enyl) benzene [49b]



49b

The quantities of chemicals used were the same as for compound **49a**. However, the mixture was refluxed for 17 hours in an oil bath at 140°C. The compound was purified by

flash column chromatography on silica gel with hexane/ethyl acetate (20/1) to obtain a yellow liquid in a yield of 46%.

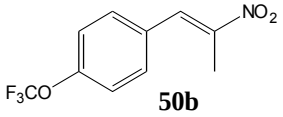
^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 8.07 (s, 1H, C=C-H), 7.44 - 4.38 (t, *J* = 8.7 Hz, 1H Ar-H), 7.27 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.19 (d, overlapped with other peaks, 1H, Ar-H), 7.17 (s, 1H, r-H), 2.34 (s, 3H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 149.1 (s, C=C, β carbon), 133.3 (Ar), 132.8 (Ar), 131.6 (Ar), 131.2 (Ar), 130.3 (Ar), 129.5 (Ar), 126.3 (s, C=C, α carbon), 123.6 (q, *J* = 272.4 Hz, 1C, CF_3), 13.8 (s, CH_3).

GC/MS *m/z* (M^+) 231.

3.4.5 Synthesis of trifluoromethoxy derivative of β-nitrostyrene

1-Trifluoromethoxy-4-(nitroprop-1-enyl) benzene [50b]



50b

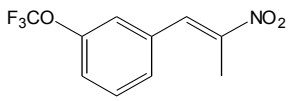
Compound **50b** was prepared using the same method as **12b**. 4-trifluoromethoxybenzaldehyde (1g, 5.3mmol), ammonium acetate (0.12g, 1.6mmol) and nitroethane (3.3g, 43.3mmol) were heated at 115°C for 5 hours. The yellow mixture was placed under high vacuum to remove excessive nitroethane and then dissolved in chloroform (10mL), washed with water (3 x 20mL) and washed again with saturated brine solution (25%, 2 x 20mL). The washed mixture was then dried over $MgSO_4$, and then concentrated under high vacuum (liquid nitrogen assisted). Yellow crystals were obtained in yield of 73% with melting point 47-48°C.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 7.98 (s, 1H, C=C-H), 7.42 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.24 (d, *J* = 8.3 Hz, 2H, Ar-H), 2.37 (s, 3H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 150.0 (s, O-C in Ar), 148.3 (s, C=C, β carbon), 131.9 (s, C=C, α carbon), 131.5 (Ar), 130.9 (Ar), 121.1 (Ar), 120.3 (q, *J* = 258.2 Hz, 1C, CF_3), 13.9 (s, CH_3).

GC/MS *m/z* (M^+) 247.

1-Trifluoromethoxy-3-(nitroprop-1-enyl) benzene [50a]



50a

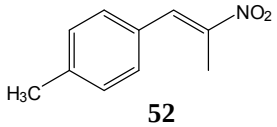
The quantities of chemicals used were the same as for compound **50b**. In the case, the mixture was refluxed for 24 hours immersed in an oil bath at 100°C. The orange yellow mixture was then extracted with ethyl acetate (20mL) and the extract washed with sodium bicarbonate ($NaHCO_3$) (3 x 20mL) and saturated brine solution (25%, 20mL). The mixture was dried over $MgSO_4$ and concentrated under high vacuum (liquid nitrogen assisted). A yellow liquid was obtained in yield of 51%. For this compound the relative amount of the *cis* and *trans* isomers are 31% and 69% respectively.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 8.05 (s, 1H, C=C-H), 7.45-7.49 (t, *J* = 8.7 Hz, 1H, Ar-H, *trans* compound), 7.40-7.36 (t, *J* = 6.6 Hz, 1H, Ar-H, cis compound), 7.31-7.28 (d, *J* = 7.2 Hz, 1H, Ar-H, *trans* compound), 7.19-7.17 (d, *J* = 7.8 Hz, 1H, Ar-H, *cis* compound), 6.49 (s, 1H, Ar-H)2.45 (s, 1H, CH_3, *trans* compound), 2.38 (s, 1H, CH_3, *cis* compound).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 149.3 (s, C-OCF_3), 149.2 (s, C=C, β carbon), 134.4 (Ar), 131.7 (s, C=C, α carbon), 130.4 (*trans* Ar), 130.0 (Ar), 128.1 (*cis* Ar), 126.2 (*trans* Ar), 124.1 (Ar), 122.6 (q, J = 258.1 Hz, 1C, OCF_3), 122.1 (Ar), 121.2 (*trans* Ar), 120.6 (*cis* Ar), 19.9 (s, 1C, *cis* CH_3), 13.9 (s, 1C, *trans* CH_3).

GC/MS *m/z* (M^+) 247.

1-Methyl-4-(nitroprop-1-enyl) benzene [52]



52

Method B was used to synthesize this compound. 4-methyl-benzaldehyde (4.00g, 33.3mmol) was dissolved in nitroethane (3.12g, 33.3mmol, 25% excess), ammonium acetate (5.13g, 66.6mmol) and glacial acetic acid (10mL) added and the mixture refluxed for 3 hours in an oil bath at 110°C. The green brown coloured mixture was then chilled and de-ionized water (15mL) was then added. The product was then dissolved in hot ethanol (95%, 2ml) and chilled for an hour to obtain the recrystallized product as light yellow crystals afterward Compound **52** (4.72g, 80% of theoretical yield). The melting point was 54-55 °C[154].

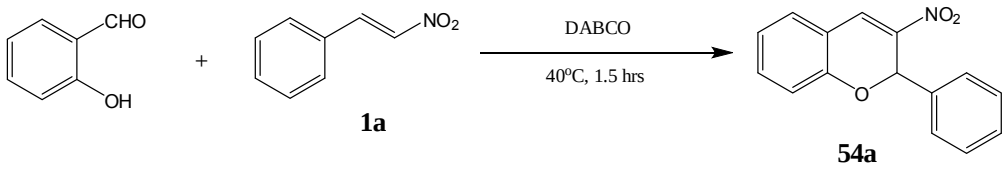
^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 7.97 (s, 1H, H-C=C), 7.46 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.17 (d, *J* = 8.0 Hz, 2H, Ar-H), 2.37 (s, 3H, C-H), 2.31 (s, 3H, C-H, *Z*).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 146.5 (C=C, β carbon), 140.9 (C=C-CH_3, carbon in the ring), 133.8 (C=C, α carbon), 130.2 (Ar), 129.7 (Ar), 129.5 (Ar), 21.3 (CH_3, methyl carbon connected to the ring), 14.1 (CH_3, connectedβ carbon)

GC/MS *m/z* (M^+) 177.

3.4.6 Synthesis of 3-nitrochromene derivatives

3-Nitrochromene [54a]

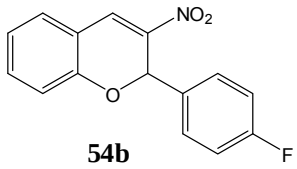


1a **54a**

The synthesis method was based on that of Yan *et al.*[187] A mixture of compound **1a** (0.5g, 3.4mmol) and salicylaldehyde (4.1g, 33.6mmol,) was swirled until the solution became

homogeneous, then a catalytic amount of DABCO (0.38g, 3.4mmol) was added to the mixed solution. The mixture was refluxed (under 40°C) for 1.5 hours and then 20mL of 5% hydrochloric acid (HCl) was added to the solution. Organic material was extracted using dichloromethane (3 x 60mL) and then dried over $MgSO_4$, and filtered. Dichloromethane was removed under high vacuum. Purification of the compound was done by silica gel flash coloumn chromatography (ethyl acetate: hexane = 1: 50). A yellow-orange solid was obtained in yield of 43% and which had a melting point of 88-90°C (Lit. 88-89℃)[187]. **^{1}H NMR** and **^{13}C NMR** spectra are consistent with Yan *et al*. **GC/MS** *m/z* (M^+) 253.

3-Nitrochromene with *para* substitution of fluorine on phenyl ring [54b]



54b

A similar procedure was used to obtain compound **54b**. The quantities of chemicals used were: compound **1b** (0.23g, 1.4mmol), salicylaldehyde (1.68g, 13.8mmol) and DABCO (0.15g, 1.4mmol). The reaction was carried out under the same reaction conditions and the purification was as for **54a**. A yellow solid was obtained in yield of 55% and which had melting a point of 88-89°C.

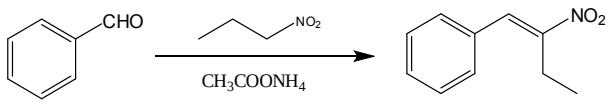
^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 7.98 (s, 1H, C=αC-H), 7.30-7.24 (m, due to peaks are overlapping each other, 4H, Ar-H), 6.96-6.89 (due to peaks are overlapping each other, 3H, Ar-H), 6.80-6.77 (d, *J* = 8.4 Hz, 1H, Ar-H), 6.47 (s, 1H, O-C-H).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 164.9-161.6 (d, *J* = 249.1 Hz, 1C, Ar-C-F), 153.3 (Ar), 141.0 (s, C=C=NO_2), 134.4 (s, 2C, Ar-C), 132.7 (s, 1C, C-Ar-F), 130.4 (Ar), 129.3 (Ar), 129.0 (Ar), 122.6 (Ar), 117.8 (C=C-C in between of two Ar), 117.3 (Ar), 115.8 (Ar), 73.5 (s, 1C, O-C-Ar-F).

GC/MS *m/z* (M^+) 271.

3.4.7 Synthesis of β-ethyl- β-nitrostyrene

2-Nitrobut-1-enyl- benzene [55]



55

The method of Kawai *et al*.[188] was used. A mixture of benzaldehyde (2.12g, 20mmol), ammonium acetate (1.54g, 20mmol) and 1-nitropropane (39.9g, 448.5mmol) was refluxed at 110℃ overnight (18 hours). The excess 1-nitropropane was removed under high vacuum and after addition of water (30mL), the organic materials were extracted with ethyl acetate (3 x 30mL) and the extract then dried over $MgSO_4$. The combined extracts were filtered and concentrated under high vacuum. The product was purified by silica gel column chromatography with hexane/ethyl acetate (20: 1) to obtain a yellow liquid [**55**] in yield of 60%.

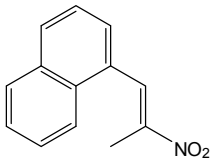
^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 8.03 (s, 1H, C=C-H), 7.47-7.45 (m, 5H, Ar-H), 2.92-2.85 (q, *J* = 7.5 Hz, 2H, CH_2), 1.32-1.27 (t, *J* = 7.5 Hz, 3H, CH_3)[188].

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 153.3 (s, C=C, β carbon), 133.1 (s, C=C, α carbon), 132.3, (Ar), 129.9 (Ar), 129.6 (2C, Ar), 129.0 (2C, Ar), 20.7 (s, CH_2), 12.5 (s, CH_3).

GC/MS *m/z* (M^+) 177.

3.4.8 Synthesis of nitro-naphthalene derivatives

1-(Nitroprop-2-enyl) naphthalene [53a]



53a

Method B was applied for this reaction and likewise for compound **53b**. A mixture of 1-naphthaldehyde (1g, 6.4mmol), ammonium acetate (0.99g, 12.8mmol) and glacial acetic acid (3mL) in nitroethane (0.6g, 8.0mmol) was refluxed for 2 hours in an oil bath at 100°C. The orange coloured mixture was then chilled and de-ionized water (6mL) was then

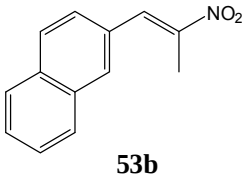
added to the orange mixture and the product which precipitated was then recrystallized from 95% ethanol. After washing with cold 95% ethanol a light yellow solid, **53a**, was obtained in yield 49% and which had a melting point of 62-64°C[189].

^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): Due to multiplets occurred in the spectra because of the protons in both aromatic ring overlapped to each other, the integration of the proton will be used to identify the peaks.8.62 (Integration of ^{1}H: 1, αC-H), 7.98-7.87 (Integration of ^{1}H: 3, Ar-H), 7.64-7.51 (Integration of ^{1}H: 4, Ar-H), 2.39 (Integration of ^{1}H: 3, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 149.3 135.7 (s, C=C, β carbon), 133.5 (Ar), 131.9 (Ar), 131.4 (s, C in between two rings), 130.2 (s, C=C, α carbon), 129.7 (s, C in between two rings), 128.8 (Ar), 127.1 (s, 2C, Ar), 126.6 (Ar), 125.1 (Ar), 124.1 (Ar), 14.1 (s, 1C, CH_3).

GC/MS *m/z* (M^+) 213.

2-(Nitroprop-2-enyl) naphthalene [53b]



53b

Quantities of chemicals used were: 2-naphthaldehyde (0.5g, 3.2mmol), nitroethane (0.3g, 4.0mmol), ammonium acetate (0.49g, 6.4mmol) and glacial acetic acid (3mL). An orange-yellow solid was obtained in yield of 41% and melting point 90-91℃[190].

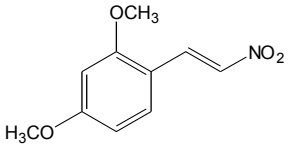
^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): Same as **53a**, the integration of the proton will be used to identify the peaks. 8.20 (Integration of ^{1}H: 1, αC-H), 7.88-7.79 (Integration of ^{1}H: 4, Ar-H), 7.49-7.43 (Integration of ^{1}H: 3, Ar-H), 2.79 (Integration of ^{1}H: 3, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 147.8 (s, C=C, β carbon), 133.7 (Ar), 133.5 (Ar), 133.0 (s, C in between two rings), 130.5 (s, C=C, α carbon), 129.8 (s, C in between two rings), 128.6 (Ar), 128.4 (Ar), 127.7 (Ar), 127.6 (Ar), 126.9 (Ar), 126.3 (Ar), 14.2 (s,1C, CH_3).

GC/MS *m/z* (M^+) 213.

3.4.9 Materials to synthesize the novel compound

2,4-Dimethoxy- β-nitrostyrene [1c]



1c

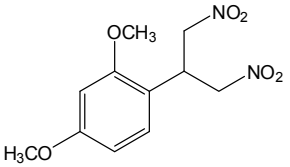
The method to make this compound was based on Fierro *et al.*[191] and also for the other dinitro compound [56b]. A mixture of 2,4-dimethoxybenzaldehyde (20g, 120.4mmol), n-butylamine (12.1 mL) and glacial acetic acid (120 mL) in nitromethane (14.7g, 240.8mmol) was refluxed for 1.5 hours at 110℃. The dark brown mixture was evaporated by high vacuum to remove the acid and water (more than 20 mL) was added to the neutralized mixture which became a yellow colour. Compound **1c** was then filtered and recrystallized in methanol to form yellow solids obtained in yield 64% and melting point of 101-104℃[192].

^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 8.10-8.06 (d, *J* = 13.5 Hz, 1H, C=αC-H), 7.85-7.80 (d, *J* = 13.5 Hz, 1H, C=βC-H), 7.39-7.37 (d, *J* = 8.4 Hz, 1H, Ar-H), 6.57-6.54 (d, *J* = 8.6 Hz, 1H, Ar-H), 6.49 (s, 1H, Ar-H), 3.93 (s, 6H, O-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 164.2 (s, 4-C-O in Ar), 161.2 (s, 2-C-O in Ar), 136.0 (s, C=C, α carbon), 135.7 (s, C=C, β carbon), 134.3 (Ar), 112.4 (Ar), 105.9 (Ar), 98.6 (Ar), 55.6 (s, CH_3).

GC/MS *m/z* (M^+) 209.

2-[2´,4´-Dimethoxybenzene]-1,3-dinitropropanes [56b]



56b

The method to synthesize this compound was also based on Fierro *et al.*[191], the starting material **1c** (9g, 43mmol) and a base, potassium fluoride (3.2538g, 51.6mmol) were added in nitromethane (5340mmol) and refluxed with stirring at 110℃ for 1.5 hours. The orange brown mixture was then evaporated under high vacuum to remove the excess

nitromethane and the solid dissolved in ethyl acetate (100 mL). After washing with water (50 mL) and then with diethyl ether (2 x 50 mL). The combined organic extract was washed again with water (3 x 40 mL). The organic extract was dried over $MgSO_4$ the diethyl ether was removed under high vacuum to obtain a light brown solid in yield 88% with melting point of 53-56℃.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 7.06-7.02 (d, *J* = 8.1 Hz, 1H, C=C-H the *ortho* hydrogen in the ring), 6.49-6.41(broad peaks due to chemical shifting and coupling of the *para* proton and *ortho* hydrogen and little coupling occurred with *meta* hydrogen in the ring), 4.84-4.82 (d, *J* = 6.90 Hz, 4H, H-H-C next to NO_2), 4.42-4.34 (p, *J* = 14.1, 7.2, 6.9 Hz, C=C-C-H), 3.87-3.79 (s, 6H, O-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 161.4 (s, 1C, C-OCH_3), 158.1 (s, 1C, C-OCH_3), 130.6 (Ar), 114.2 (Ar), 104.8 (Ar), 99.3 (Ar), 75.6 (s, 2C, CH_2), 55.5 (s, 1C, OCH_3), 55.4 (s, 1C, OCH_3), 38.9 (s, 1C, NO_2-C-C-C-NO_2).

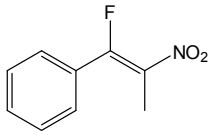
GC/MS *m/z* (M^+) 270.

3.5 Attempted synthesis of other nitro compounds

There were a few additional compounds required to be made for antimicrobial tests [**56a**, **57**, **58a** and **58b**] and also as starting materials [**59** and **60**] required for making novel compounds. All of them, except **57**, are mentioned in the literature, but none were able to be synthesized successfully. They are described below:

3.5.1 Compound with fluorine substitution on α-carbon

α-Fluoro-β-methyl-β-nitrostyrene [57]

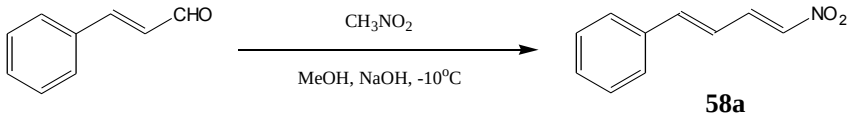


57

The synthesis procedure was based on Yusubov *et al*.[193]. To a mixture of solution of 1-phenyl-1-propyne (0.7g, 6.0mmol) and sodium nitrate (1.02g, 12.0mmol) in acetic acid (20mL), potassium fluoride (0.35g, 6.0mmol) was added when the solution was at 85°C. The orange mixture was refluxed overnight (18 hours) and the product formation was monitored by TLC. The reaction mixture was then poured into water (60mL) and extracted with diethyl ether (3 x 100mL). The organic extracts were washed with water (3 x100mL) and saturated brine solution (25%. 60mL) and dried over $MgSO_4$. Diethyl ether was removed by high vacuum. However, the orange coloured compound with some white crystals was still not pure and it could not be purified by column chromatography.

3.5.2 Compounds mentioned in literature

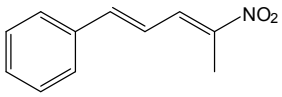
(4-Nitrobuta-1,3-dienyl)benzene [58a]



58a

Attempts were made to synthesize this compound using Method A, Method B and a literature method from Dockendorff *et al*.[194], but none of them was successful.

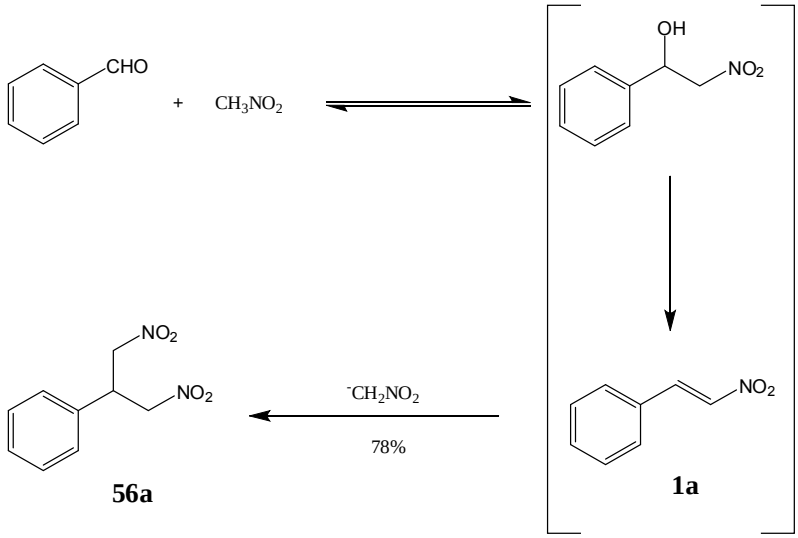
(4-Nitropenta-1,3-dienyl)benzene [58b]



58b

Methods A, B and the method from Dockendorff *et al*.[194] were used for this compound. Neither of them succeeded as well as described. However, method from RodrÃguez and Dolors Pujol[195] suggested another possibility to synthesize **58a** and **58b**.

(1,3-Dinitroprop-2-enyl)benzene [56a]

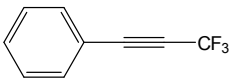


56a **1a**

Attempts were made to synthesize this compound using the methods from Ballini *et al*.[196] and Iturriaga-Vásquez *et al*.[197]. However neither method yielded the dinitro-compound. The method of Fierro *et al*.[191] to make compound **56b** may be the way to synthesize this compound

3.5.3 Attempted synthesis of starting materials

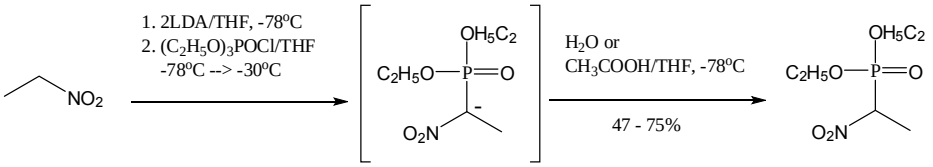
3,3,3-Trifluoro-1-phenylpropyne [59]



59

The literature method was according to Bunch and Bumgardner[198]. However, due to limited material available for the reaction, the compound could not be obtained.

Alkyl Phosphonate [60]

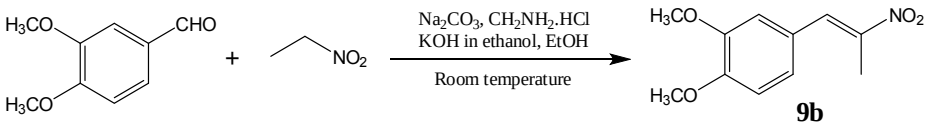


60

The method of synthesis was from Kandil *et al.*[199] on a 50% scale. A solution of lithium diisopropylamide (LDA, 1.6mL, 11mmol diisopropylamine reacted with 1.0mL, 11mmol of 2.5M n-butyllithium in hexane) in dry tetrahydrofuran (THF, 25mL) was prepared under nitrogen at -78°C. Nitroethane (0.412g, 5.5mmol) in dry THF (50mL) was then added dropwise over half an hour. The mixture was stirred for another half hour and a solution of diethyl chlorophosphate (0.95g, 5.5mmol) in dry THF (5mL) was added dropwise over 15 minutes and the mixture was stirred continuously for an additional 3 hours. The solution was warmed to -30°C and stirred for another 2 hours. After that the mixture was cooled back to -78°C and acetic acid (1.32g, 22mmol) in dry THF was added dropwise to quench the mixture and stirring was maintained for one hour at -78°C. The mixture was then gradually warmed to room temperature. The mixture was diluted with water (50mL) and the organic materials were extracted with ethyl acetate (3 x 25mL), washed with saturated brine solution (25%, 2 x 14mL) and dried with $MgSO_4$. The combined extracts were filtered and concentrated under high vacuum. Further purifications were achieved by dissolving the compound in ether (20mL) and then extracting with the saturated sodium carbonate solution (3 x 20mL). The combined aqueous extracts were washed with diethyl ether (2 x 20mL) and acidified to pH 7 by glacial acetic acid and to pH 2 with 10% aqueous hydrochloric acid. The remaining compound was then extracted with diethyl ether (3 x 50mL), filtered and dried over $MgSO_4$. The solvent was removed in high vacuum, but the desired product could not be obtained, which was shown by ^{1}H NMR and GC/MS.

3.5.4 Other compounds synthesized by Professor Hugh Cornell

1,2-Dimethoxy-4-(2-nitroprop-1-enyl)benzene [9b]



9b

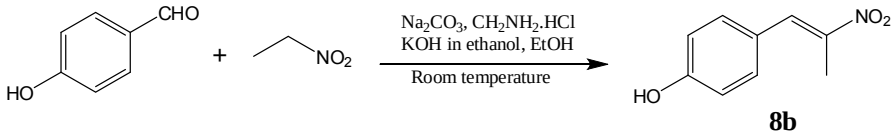
Compound **9b** was prepared by Method A. A mixture of 3,4-dimethoxybenzaldehyde (1.66g, 10mmol), nitroethane (1g, 13.3mmol), anhydrous sodium carbonate (0.2g, 2mmol), methylamine hydrochloride (0.11g, 1.5mmol) potassium hydroxide (0.05g, 0.9mmol, dissolved in 1.0 mL ethanol) and ethanol (2.5mL) was reacted at room temperature with sufficient stirring for 24 hours. After that, the mixture was diluted with water (3mL) and chilled to 5℃ for 3 hours. The crude product was filtered and air dried, recrystallized with 95% ethanol and air dried. The final product was yellow and crystalline with yield of 27% and a melting point of 71-72°C[22].

^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 7.99 (s, 1H, C=C-H), 6.94 (s, 1H, Ar-H), 7.03-7.00 (d, *J* = 8.1 Hz, 1H, Ar-H), 6.88-6.85 (d, *J* = 9.3 Hz, 1H, Ar-H), 3.85 (s, 6H, OCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 150.7 (s, C3 of C-OCH_3 in the ring), 149.1 (s, C4 of C-OCH_3 in the ring), 145.9 (s, C=C, β carbon), 133.8 (Ar), 125.0 (s, C=C, α carbon), 124.0 (Ar), 113.0 (Ar), 111.2 (Ar), 56.0 (s, 2C, OCH_3), 14.1 (CH_3).

GC/MS *m/z* (M^+) 223.

1-Hydroxy-4-(2-nitroprop-1-enyl)benzene [8b]



8b

Method A was also used for preparation **8b**. A mixture of 4-hydroxybenzaldehyde (2.44g, 20mmol) dissolved in absolute ethanol (3mL), nitroethane (2g, 26.6mmol), anhydrous sodium carbonate (0.3g, 3mmol) and methylamine hydrochloride (0.15g, 2.2mmol), was prepared and a solution of potassium hydroxide (0.05g, 0.9mmol, dissolved in 1.0 mL ethanol) added with thorough mixing. The mixture was reacted at room temperature with

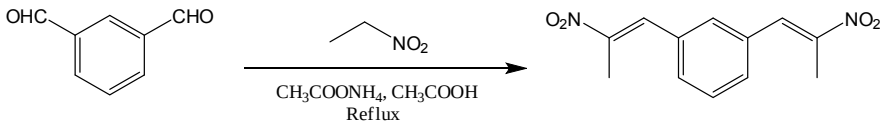
sufficient stirring for 48 hours. Yellow crystals were obtained after chilling to 5℃ for 2 hours. The crude product was filtered and recrystallized from 95% ethanol to yield 1g of product (50% of the theoretical yield) and melting point 121-122℃[200].

^{1}H NMR (300 MHz, DMSO): δ_H (ppm): 8.03 (s, 1H, C=C-H), 7.50-7.47 (d, *J* = 8.7 Hz, 1H, Ar-H), 6.90-6.87 (d, 8.7 Hz, 1H, Ar-H), 2.40 (s, 3H, CH_3).

^{13}C NMR (75 MHz, DMSO): δ_C (ppm): 159.7 (s, C-OH), 144.4 (s, C=C, α carbon), 133.7 (s, C=C, β carbon), 132.8 (Ar), 122.5 (Ar), 115.9 (Ar), 13.9 (CH_3).

GC/MS *m/z* (M^+) 179.

1,4-Bis-(2-nitro-propenyl)-benzene [51]



51

Method B was applied to produce this compound. A stirred mixture of terephthaldicarboxaldehyde (1.34g, 10mmol), nitroethane (1.8g, 24mmol) and ammonium acetate (2g, 26mmol) in glacial acetic acid (3mL) was refluxed for 1 hour in an oil bath at 100°C. A yellow precipitate formed which was washed with water and extracted with ethyl acetate (2 x 15mL). The orange solution was dried over $MgSO_4$ and then concentrated under high vacuum. A semi-solid was formed with melting point 90-95℃. The semi-solid was then recrystallized with ethanol (95%) and produced a yellow solid in yield 18% with melting point 99-101℃[201]. The method from Fierro *et al*.[191] is potentially another good method to synthesize this compound.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 8.10 (s, 2H, C=C-H), 7.54 (s, 4H, Ar-H), 2.50 (s, 6H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ_C (ppm): 148.7 (s, C=C, β carbon), 133.8 (Ar), 132.4 (s, C=C, α carbon), 130.3 (s, 4C in Ar), 14.1 (s, 2C, CH_3).

GC/MS *m/z* (M^+) 248

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Appendix

Compound no.: Name

25: 4-fluorobut-2-ynyl 2-(3-fluoro-4-methylphenyl)-3,3-dimethylbutanoate

26: 4-(2,3-difluorophenyl)-6-(4-fluorobut-2-ynyloxy)pyrimidine

27: 3-(4-fluorobut-2-ynyloxy)-5-phenyl-1,2,4-thiadiazole

28: 1-(4-(3-chlorophenyl)-4-fluorobut-2-ynyloxy)-4-fluoro-2-methoxybenzene

29: 6-(4-chlorophenyl)-2-(4-fluoropent-2-ynyl)-4,5-dihydropyridazin-3(2*H*)-one

30: methyl-2-chloro-5-(3-fluoro-3,4-dimethylpent-1-ynyl)benzylcarbamate

31:(E)-methyl-3-methoxy-2-(2-methyl-5-(3,4,4,4-tetrafluoro-3-methylbut-1-ynyl)phenoxy)acrylate

32: 4′-(3-fluorobut-1-ynyl)biphenyl-2-amine

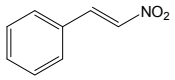
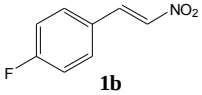
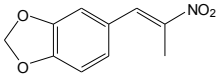
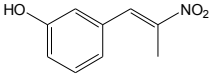
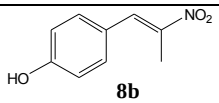
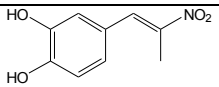
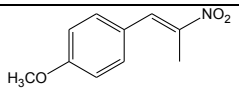
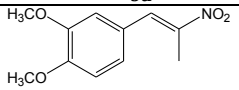
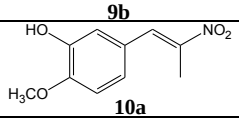
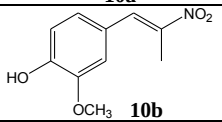
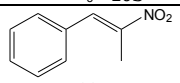
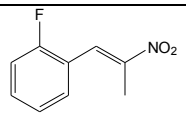
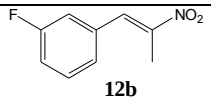
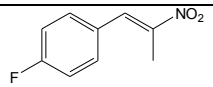
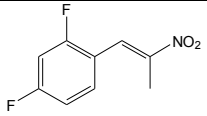
33: methyl 2-(7-(3-fluoro-3-methylbut-1-ynyl)naphthalen-1-yl)-3-methoxypropanoate

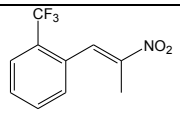
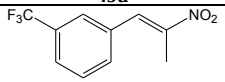
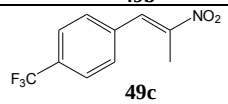
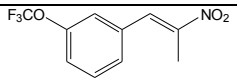
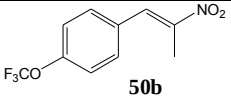
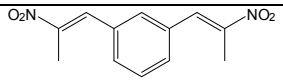
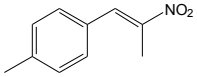
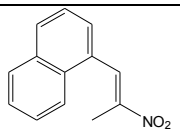
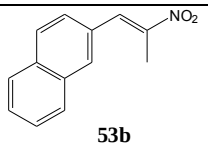
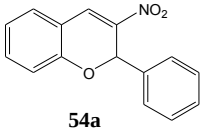
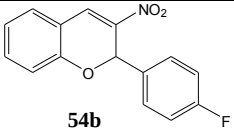
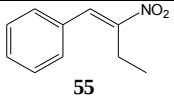
34: 2-((5-(3-fluorobut-1-ynyl)thiophen-2-yl)ethynyl)benzo[*b*]thiophene

35: 6-chloro-4-cyclopropyl-4-(3-fluoroprop-1-ynyl)-3,4-dihydroquinazolin-2(1*H*)-one

36:N-(4-chlorobenzyl)-8-(3-fluoroprop-1-ynyl)-1-methyl-6-(morpholinomethyl)-4-oxy1,4-dihydroquinoline-3-carboxamide

Table of all structures with measured logP and calculated logP

Structure	Measured logP	Calculated logP
 **1a**	1.70	2.13
 **1b**	1.79	2.27
 **7**	2.55	1.75
 **8a**	2.16	1.90
 **8b**	2.18	1.90
 **8c**	2.04	1.59
 **9a**	2.68	2.04
 **9b**	2.40	1.88
 **10a**	1.61	1.74
 **10b**	2.27	1.74
 **11**	2.05	2.20
 **12a**	2.14	2.34
 **12b**	1.15	2.34
 **12c**	2.00	2.34
 **12d**	1.81	2.49

 **49a**	1.78	3.08
 **49b**	1.48	3.08
 **49c**	1.83	3.08
 **50a**	1.26	3.63
 **50b**	2.19	3.63
 **51**	2.74	2.43
 **52**	2.66	2.71
 **53a**	3.17	3.19
 **53b**	3.41	3.19
 **54a**	2.45	3.30
 **54b**	2.63	3.44
 **55**	1.84	2.72